DEPARTMENT OF THE ARMY
US ARMY PUBLIC HEALTH COMMAND (PROVISIONAL)
5158 BLACKHAWK ROAD
ABERDEEN PROVING GROUND MD 21010-5403

MCHB-IP-THE

MEMORANDUM FOR Environmental Acquisition and Logistics Sustainment Program (AMSRD-MSF/Mr. Erik Hangeland), US Army Research, Development and Engineering Command, 5183 Blackhawk Road, Aberdeen Proving Ground, MD 21010-5424

SUBJECT: Toxicology Study No. 87-XE-0BMEa-10, Protocol No. 0BME-30-09-03-01, Effects of Oral TAG 1-MeATN02 (Triaminoguanidinium-1-methyl-5-nitriminotetrazolate – TAG-MNT/K-26) Exposure to Female Rats *(Rattus norvegicus)*, August 2010

1. Five copies of the subject report with Executive Summary are enclosed.

2. The point of contact is Dr. Larry Williams, Project Manager, at (410) 436-7159, DSN 584-7159 or FAX (410) 436-8258. He may also be reached by electronic mail at larry.williams45@us.army.mil.

FOR THE COMMANDER:

CINDY A. LANDGREN
LTC, VC
Director, Toxicology

USAPHC

U.S. ARMY PUBLIC HEALTH COMMAND (Provisional)

5158 Blackhawk Road, Aberdeen Proving Ground, Maryland 21010-5403

TOXICOLOGY STUDY NO. 87-XE-0BMEa-10
PROTOCOL NO. 0BME-30-09-03-01
EFFECTS OF ORAL TAG 1-MeATN02 (TRIAMINOGUANIDINIUM-1-METHYL-5-NITRIMINOTETRAZOLATE – TAG-MNT/K-26) EXPOSURE TO FEMALE RATS *(RATTUS NORVEGICUS)*

AUGUST 2010

Toxicity Tests: 40-5k1

ACKNOWLEDGEMENTS

The authors would like to acknowledge the support and encouragement provided to this effort by Mr. Erik Hangeland and Ms. Maryalice Miller, present and former Directors, respectively, of the U.S. Army Research, Development and Engineering Command, Environmental Acquisition and Logistics Sustainment Program. We also thank Dr. John Beatty of the Environmental Quality Technology Program, Pollution Prevention Team for assistance. Several personnel contributed to the completion of this study through care of animals, participation in animal dosing, data collection and necropsy; these include Pat Beall, Major Anne MacLarty, Becky Kilby, Jason Williams, Dr. Will McCain, Dr. Valerie Adams, Terry Hanna, John Houpt, Mark Way, and Dr. Emily LaFiandra. Particular acknowledgment goes to Martha Thompson for collecting LABCAT® data and generating the appropriate reports, and Matt Bazar for performing the blood chemistry and hematology assays and preparing the reports.

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US Army Public Health Command (Provisional) [USAPHC(Prov)]
[formerly US Army Center for Health Promotion and Preventive Medicine (USACHPPM)] Directorate of Toxicology, Health Effects Research Program
5158 Blackhawk Rd., Aberdeen Proving Ground, MD 21010-5403

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14. ABSTRACT

The objective of this research is to determine the oral LD50 of Triaminoguanidinium-1-methyl-5nitriminotetrazolate (TAG-MNT), and to determine if adverse effects occur from a 14-day repetitive oral exposure regime of TAG-MNT in the female rat, i.e., derive the no-observable-effect-level (NOAEL) and lowest-observed-effect-level (LOAEL). TAG-MNT is not acutely toxic at the limit dose, but as early as 14-days of daily oral exposure causes liver toxicity and death. The primary target organ is the liver; TAG-MNT causes centrilobular hypertrophy, vacuolization and increased liver weight. In the higher dose groups liver pathology is correlated with decreased food consumption, body weight loss, and death. The LOAEL from oral exposure to TAG-MNT for 14-days, as determined from this study, is 500 mg/kg-day based on significant adverse event of increased kidney and liver weight ratios and adverse histological alterations in the liver. The NOAEL is therefore determined to be 250 mg/kg-d where only mild adaptive hepatic vacuolization was observed in a subset of exposed individuals.

15. SUBJECT TERMS

rat, Triaminoguanidinium-1-methyl-5-nitriminotetrazolate (TAG-MNT), 14 day oral toxicity

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ATTN: AMSRD-MSF
Environmental Acquisition and Logistics Sustainment Program
Aberdeen Proving Ground, MD 21010

Study Title

Toxicology Study No. 87-XE-0BMEa-10
Protocol No. 0BME-30-09-03-01
Effects of Oral TAG 1-MeATN02
(Triaminoguanidinium-1-methyl-5-nitriminotetrazolate – TAG-MNT/K-26)
Exposure to Female Rats *(Rattus norvegicus)*

Authors

Larry Williams, Ph.D. and Lee C.B. Crouse

Report Completed

August 2010

Performing Laboratory

U.S. Army Public Health Command (Provisional)
(formerly U.S. Army Center for Health Promotion and Preventive Medicine)
Directorate of Toxicology
Health Effects Research Program
MCHB-TS-THE
Aberdeen Proving Ground, MD 21010-5403

Laboratory Project ID

Toxicology Study No. 87-XE-0BMEa-10

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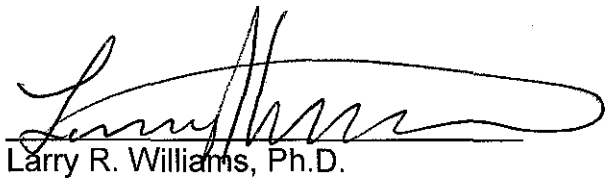

8-9-10

Larry R. Williams, Ph.D.
Biologist
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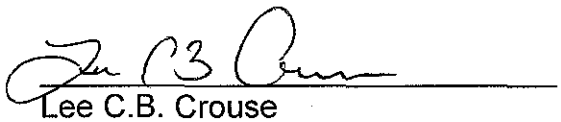
Date



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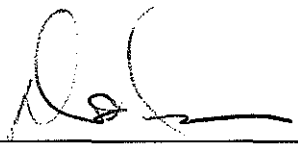

8-24-10

Mark S. Johnson, Ph.D., D.A.B.T.
Manager
Health Effects Research Program

Date

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The study described in this report was conducted in compliance with Title 40, Code of Federal Regulations (CFR), Part 792, Good Laboratory Practice Standards. No deviations from the aforementioned regulation affected the quality or integrity of the study or the interpretation of the results.

Larry R. Williams, Ph.D.
Study Director
Health Effects Research Program

8-24-10
Date

DEPARTMENT OF THE ARMY
US ARMY PUBLIC HEALTH COMMAND (PROVISIONAL)
5158 BLACKHAWK ROAD
ABERDEEN PROVING GROUND MD 21010-5403

MCHB-TS-THE

EXECUTIVE SUMMARY
TOXICOLOGY STUDY NO. 87-XE-0BMEa-10
PROTOCOL NO. 0BME-30-09-03-01.
EFFECTS OF ORAL TAG 1-MeATN02
(TRIAMINOGUANIDINIUM-1-METHYL-5-NITRIMINOTETRAZOLATE – TAG-MNT/K-26) EXPOSURE TO FEMALE RATS *(RATTUS NORVEGICUS)*
AUGUST 2010

1. PURPOSE. The objective of this study was to determine the oral LD_{50}, 95 percent confidence intervals and slope from oral administration of TAG-MNT (K-26), and to determine if adverse effects occur from a 14-day repetitive oral exposure regime of TAG-MNT in the female rat, i.e., derive the no-observable-effect-level (NOAEL) and lowest-observed-effect-level (LOAEL).

2. CONCLUSIONS.

a. TAG-MNT was not acutely toxic at the limit dose, but as early as 14-days of daily oral exposure caused liver toxicity and morbidity. The primary target organ was the liver; TAG-MNT caused centrilobular hypertrophy, vacuolization and increased liver weight. In the higher dose groups liver pathology was correlated with decreased food consumption, body weight loss, and morbidity.

b. Measured signs of toxicity in the 14-day study at dosages of 500 milligrams per kilograms per day (mg/kg-day) and above included decreased food consumption as well as alterations in the kidney and liver weight ratios, when compared to controls. Histopathologic evidence of adverse events was observed in a dose-response manner beginning with 250 mg/kg-d.

c. The LOAEL from oral exposure to TAG-MNT for 14-days, as determined from this study, is 500 mg/kg-day based on significant adverse event of increased kidney and liver weight ratios and adverse histological alterations in the liver. The NOAEL is therefore determined to be 250 mg/kg-d where only mild adaptive hepatic vacuolization was observed in a subset of exposed individuals.

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EFFECTS OF ORAL TAG 1-MeATN02 (TRIAMINOGUANIDINIUM-1-METHYL-5-NITRIMINOTETRAZOLATE – TAG-MNT/K-26) EXPOSURE TO FEMALE RATS *(RATTUS NORVEGICUS)*
AUGUST 2010

1. REFERENCES. See Appendix A for a listing of references used in this report.

2. PURPOSE. The objective of this study was to determine the oral LD_{50}, 95 percent confidence intervals and slope from oral administration of TAG-MNT (K-26), and to determine if adverse effects occur from a 14-day repetitive oral exposure regime of TAG-MNT in the female rat, i.e., derive the no-observable-effect-level (NOAEL) and lowest-observed-effect-level (LOAEL).

3. AUTHORITY. MIPR No. 0BDAT4D100. This toxicology study addresses, in part, the environmental safety and occupational health (ESOH) requirements outlined in Army Regulations (AR) 200-1 , AR 40-5 , and AR 70-1; Department of Defense Instruction (DoDI) 4715.4 (DOD 4715.4), and Army Environmental Research and Technology Assessment (AERTA, 2007) requirement A (3.3.c), *Compliant Ordnance Lifecycle for Readiness of the Transformation and Objective Forces.* This program is under the direction of the U.S. Army Research, Development and Engineering Command (USARDECOM) Environmental Acquisition Logistics & Sustainment Program and Environmental Quality Technology (EQT) Pollution Prevention.

4. BACKGROUND.

a. Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX, Royal Demolition explosive), an important high explosive used in many formulations in a variety of weapon systems,, has been found in the ground water migrating off military lands and causing halting of range operations, affecting operational readiness. The U.S. Environmental Protection Agency (USEPA) has developed an acute oral minimum risk level (MRL) of 60 micrograms per kilograms per day (µg/kg-day) based on epileptiform seizure neurotoxicity in humans and rodents (Stone et al., 1969; Burdette et al., 1988; Kasuske et al., 2009; Williams et al., 2010), and a reference dose (RfD) of 3 µg/kg-day based on prostatic inflammation in rodents. RDX is also classified as a possible carcinogen (Lish et al., 1984a; Parker et al., 2006).

b. Although military operations require the use of unique substances, such as energetics, explosives, and propellants, encroachment on military installations and recently discovered health concerns of these substances can halt training, affect military readiness and result in costly remedial alternatives. Historically, military munitions were developed based strictly on their effectiveness on the battlefield. Formal toxicity

screening was not required, and the environmental impacts were discovered long after implementation, leading to costly remediation efforts. Recently, the need to balance effectiveness with sound environmental stewardship has become a greater priority. Toxicity evaluations are now being performed during the research, development, testing, and evaluation (RDT&E) stage rather than following acquisition when design modifications are more costly. Determinations using toxicity data with useful fate and transport information will help provide guidance on the possible environmental aspects of fielding of munitions and on training guidance prior to use.

c. The Army EQT Ordnance Environmental Program (OEP) is dedicated to finding replacements for RDX that will reduce or eliminate the health risks from environmental exposure and will reduce adverse ESOH effects and effects of RDX on readiness and costs associated with training (USACHPPM, 2007). By identifying unacceptable ESOH effects early in the acquisition process, unacceptable replacements can be identified and unnecessary budget expenditures can be greatly reduced.

d. In collaboration with USARDECOM, personnel in the Department of Chemistry and Biochemistry, Ludwig-Maximilian University, Munich, Germany, have synthesized several energetic compounds as possible replacements for RDX. TAG-MNT is one of the lead energetics from this laboratory (Hammer et al., 2005; Klapotke et al., 2008a; Klapotke et al., 2008b).

e. This document reports the conduct, findings, and conclusions of a progressive series of two oral toxicity studies performed with TAG-MNT in laboratory female rats. The series consisted of a Sequential Stage-Wise Probit, (SSWP) acute test and a 14-day repeated dose study. Such investigations identify effect levels and define target organs to determine how the mammalian toxicity of the material compares to currently fielded explosives.

f. The following table identifies the critical dates of the acute and 14-day studies.

Table 1. Critical Dates of Acute and 14-Day Studies

Critical Event	Date of Event
Animal Use Protocol Approved	03/28/2009
SSWP Animals Received	09/30/2009
Study Start	10/07/2009
Experimental Start	10/08/2009
SSWP Necropsies	10/22/2009, 10/27/2009, 10/29/2009
14-Day Animals Received	11/18/2009
14-Day Study Start	11/24/2009
14-Day Necropsies	12/8/2009, 12/9/2009, 12/10/2009
Experimental Completion	12/10/2009
Study Completion	08/15/2010

5. MATERIALS.

a. Test Substances.

(1) The TAG-MNT (CAS # not assigned), 1376-00-D02-0746, was obtained from the Army Research Development and Engineering Center (ARDEC) Picatinny Arsenal, NJ 07806. The lot number was identified as RDD09A004E001 with a purity of 98-99 weight percent. The material was shipped as a 600-gram sample of dry granules. The Chromatographic Analysis Division (Explosives Team), Directorate of Laboratory Sciences (DLS), U.S. Army Public Health Command (Provisional) (USAPHC (Prov)) developed an analytical method using the neat material from ARDEC as the standard. Methylcellulose (CAS # 9004-67-5) was purchased from Fisher Scientific. (Fairlawn, NJ 07410; lot number 037690). Tween 80 (CAS # 9005-65-6) was purchased from Fisher Scientific (Fairlawn, NJ 07410; lot no. 032097).

(2) In the 14-day repeated dose study, intended doses of 2000 milligrams per liter (mg/L) were above the room temperature water solubility of TAG-MNT (~1000 mg/L). In addition, although standard protocol for the 14-day study is to store compound solutions at 4 degrees centigrade (°C) to preserve stability, preliminary experiments determined that such storage resulted in formation of large crystals of the saturated TAG-MNT solutions, crystals too large to pass through the 18-gauge oral gavage needle needed for dosing. Thus, solutions and 2000 mg/L suspensions of TAG-MNT were stored at room temperature (RT) for the duration of the study. Three dosing solutions were prepared with intended concentrations of 2000 mg/L, 500 mg/L, and 125 mg/L. The solvent for the 14-day repeated dose study was chosen to be a combination of 1 percent methylcellulose, 0.2 percent Tween 80, in tap water. The homogeneity of the 2000 mg/L dosing solution was verified by taking 100 microliter samples at the top, middle, and bottom for analysis using high-performance liquid chromatography (HPLC) with a ultraviolet (UV) detector at a wavelength of 313 nanometers (nm), with an eluent mixture of 75 percent methanol 25 percent water. In addition, samples of the TAG-MNT solutions were taken and analyzed to verify compound concentrations and the 125 mg/L solution was sampled every 3-4 days (twice per week) for a period of 3 weeks to ensure that the TAG-MNT concentration would remain stable after storage at RT throughout the testing period. In addition, samples of all three dosing solutions were taken at the start of the study to determine exact dosing volumes and at the end of the study to verify stability of the dosing solutions at RT.

b. Animals.[1]

[1] Research was conducted in compliance with DOD and Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council. National Academy Press, Washington, D.C. 1996. The studies reported herein were performed in animal facilities fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

(1) All studies were conducted using young adult female Sprague-Dawley rats obtained from Charles River Laboratories (Wilmington, MA). At the time of their arrival, the animals for the acute study were 8-weeks old, and the animals for the 14-day study were 6-weeks old. The Attending Veterinarian examined the animals and found them to be in acceptable health. The animals were quarantined for a minimum of 5 days after their arrival in this facility. All rats were maintained in a temperature-, relative humidity-, and light-controlled room. The conditions were 64-79 degrees Fahrenheit (°F), 30 percent to 70 percent relative humidity with a 12-hour light/dark cycle (USACHPPM., 2006). A certified pesticide-free rodent chow (Harlan Teklad®, 8728C Certified Rodent Diet) and drinking quality tap water were available *ad libitum* cycle (USACHPPM., 2006). Rats were housed individually in suspended polycarbonate boxes with Harlan Sani-Chip® bedding. Each rat was uniquely identified by number using cage cards only for the acute study and both cage cards and microchip implants (BioMedic Data Systems, Inc., Seaford, Delaware) for the 14-day study. (Teklad® is a registered trademark of Harlan, Teklad; Harlan® Sani-Chip is a registered trademark with P.J. Murphy Forest Products Corporation.)

c. Contract Studies. Carol L. Meschter, DVM, Ph.D., DAVCP (Comparative Biosciences, Inc., 786 Lucerne Drive, Sunnyvale, CA 94085) performed the histopathological evaluations for the 14-day study under commercial contract W91ZLK-08-P-1897.

d. Quality Assurance. The USAPHC (Prov) Quality Systems Office audited critical phases of this study. Appendix B provides the dates of these audits along with the audited phase and date reported to Management and the Study Director.

e. Study Personnel. Appendix C contains the names of persons contributing to the performance of these studies.

6. METHODS.

a. Acute Study (Sequential Stage-Wise Probit, SSWP).

(1) The objective of this phase of the study was to determine the median acute oral lethal dose (LD_{50}) of TAG-MNT in the female Sprague-Dawley rat. Results are useful for relative toxicity comparisons and to determine dosage levels for the subacute (14-day) study. The general procedures of this acute study followed the USEPA Health Effects Test Guidelines for Acute Oral Toxicity (OPPTS 870.1100) (USEPA, 1998).

(2) The SSWP test was used to determine the estimated oral LD_{50} and confidence interval of TAG-MNT to the Sprague-Dawley rat (Feder et al., 1991a; Feder et al., 1991b). Tests were performed using two separate stages of dosing.

(3) All animals were fasted overnight prior to dosing and for up to 4 hours post-dosing. Based on reported toxicity of TAG-MNT in the neutral red cytotoxicity assay, the oral LD_{50} of TAG-MNT is estimated as being 900 milligrams per kilogram (mg/kg) (USACHPPM, 2008b). Doses for the first stage of the acute tests were set at 180, 270, 400, 600, 900, 1350, and 2000 mg/kg. All doses were calculated based on body weights taken immediately prior to dosing. The amount of TAG-MNT appropriate for each rat was weighed individually in a weigh pan, suspended in corn oil, and administered by oral gavage using a 16 gauge x 2-inch stainless steel gavage needle; maximum volume did not exceed 10 milliliters per kilogram (mL/kg) (USEPA, 1998).

(4) In the second stage, two groups of 3 female rats were dosed with 2000 mg/kg TAG-MNT. During the course of the acute study, it was determined that the preferred vehicle for the 14-day, sub-acute study would be 1 percent methylcellulose, 0.2 percent Tween 80, in tap water; suspension of TAG-MNT was prolonged and the TAG-MNT more soluble in the methylcellulose/Tween 80/ tap water vehicle. To rule out an effect of vehicle on possible toxicity of TAG-MNT, the corn oil vehicle was repeated coincident with a second group dosed with TAG-MNT suspended in methylcellulose.

(5) Following the administration of the test compound for each phase of the acute test the rats were observed for 14 days. All clinical signs or incidences of death were recorded on a daily basis. Individual body weights were recorded daily (5 days a week) throughout the 14-day observation period to determine recovery.

(6) Surviving animals were euthanized on day 14 and submitted for gross pathological examination. The estimated LD_{50} value was then calculated using the number of surviving animals at the end of the 14-day observation period for each stage of dosing.

b. 14-Day Oral Repeated Dose Toxicity Study.

(1) Upon evaluating the results of SSWP, a 14-day repeated dose oral toxicity study was conducted in female rats according to the Toxicology Directorate SOP for 14-day Oral Toxicity Study in Rats (USACHPPM, 2008a)

(2) Seventy female Sprague-Dawley rats, obtained at 6 weeks of age, were used for this phase of the study. Following a 5-day quarantine/acclimatization period, the animals were randomly distributed using the LABCAT® randomization program into 7 treatment groups consisting of 10 female rats each. Dosage levels were set at 0 (methyl cellulose vehicle control), 62.5, 125, 250, 500, 1000, and 2000 milligrams per kilogram per day (mg/kg-day). The animals were then divided into three evenly distributed experimental groups; the start dates for each group were staggered over a period of three days to facilitate scheduling of necropsies. (LABCAT® is a registered trademark of Innovative Programming Associates.)

(3) In an effort to obtain accurate food consumption data, three dosing solutions were prepared with intended concentrations of 2000 mg/L, 500 mg/L, and 125 mg/L. The three suspensions/solutions were used to dose each of dosing groups administering either 10 mL/kg or 5 mL/kg; dosing volume did not exceed 10 mL/kg (USEPA, 1998). The methylcellulose control animals were dosed at the same volume per body weight as the highest dosage group receiving the TAG-MNT solution 10 mL/kg. The doses were administered daily, 7 days per week (total of 14 doses) for the 14-day study. A 16 gauge x 2-inch stainless steel gavage needle was used to facilitate oral dosing. The solution/suspensions were sampled and analyzed by DLS to verify concentrations and stability prior to the first day of dosing.

(4) Body weights were recorded on days -1, 0, 1, 3, 7, and 14. Food consumption based on change in feeder weights was monitored weekly. Animals were observed daily for toxic signs and morbidity. Water consumption was not monitored during this study. All data were recorded using the LABCAT In-Life Data Collection and Reporting application (Lawrenceville, NJ).

(5) Following the 14-day study period, the rats were anesthetized with isoflurane gas. Blood was collected by intracardiac puncture, and the rats were euthanized using carbon dioxide. Clinical chemistry and hematology values were determined from all valid samples. The adrenals, brain, heart, kidneys, liver, ovaries, spleen, thymus, and uterus were removed and weighed for absolute organ weights, organ-to-body weight ratios, and organ-to-brain weight ratios. Gross necropsies were completed on all terminal animals. The following parameters, by test group, were analyzed and compared to the controls:

(a) Body weights.

(b) Weight gains.

(c) Food consumption.

(d) Absolute organ weights.

(e) Organ-to-body weight ratios.

(f) Organ-to-brain weight ratios.

(g) Hematology (Cell-Dyn 3700 Hematology Analyzer, Abbott Laboratories, Abbott Park, Illinois 60064): white blood cell count (WBC), WBC differential (percent neutrophils (NEU %N), percent lymphocytes (LYM %L), percent monocytes (MONO %M), percent eosinophils (EOS %E), percent basophils (BASO %B)), red blood cell

count (RBC), hemoglobin (HGB), hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelets (PLT), and mean platelet volume (MPV).

(h) Clinical Chemistry (VetTest 8008 Chemistry Analyzer and VetLyte Na, K, Cl Analyzer, IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, ME 04092): albumin (ALB), alkaline phosphatase (ALK P), alanine aminotransferase (ALT), blood urea nitrogen (BUN), calcium (Ca), cholesterol (CHOL), creatinine (CREA), glucose (non-fasting) (GLU), globulin (GLOB), lactate dehydrogenase (LDH), phosphorus (PHOS), total bilirubin (TBIL), total protein (TP), sodium (Na), potassium (K), and chlorine (Cl).

(i) Statistics. For variables that were measured only at the end of the study, the dose groups were compared using a one-factor analysis of variance (ANOVA). Organ-to-brain and organ-to-body-weight ratios were calculated and analyzed similarly to the other parameters measured at the end of the study with the ratio transposed by factors of 10 to make the ratios appear as numbers greater than 1. If the ANOVA was significant, Dunnett's multiple comparison test was used to determine statistical significance ($p \leq 0.05$) of individual TAG-MNT dose groups to the control group.

7. RESULTS.

a. Analytical Chemistry. The analytical chemistry results from both the acute and 14-day studies are contained in Appendix D. Overall the solutions were homogenous and stability remained within acceptable limits for a 14-day period. The results from the homogeneity study indicated that the method of suspension preparation of the highest dose group over estimated the actual concentration of compound. Thus, the dosing solutions for the subacute study were deliberately made 20 percent more concentrated to ensure that the volume of each dose to the animals did not exceed 10 mL/kg. The results of the dosing solution concentration samples were consistent with the calculated nominal concentrations. Adjustments were made to the dosing volumes based on the analytical concentrations of the solutions/suspensions. TAG-MNT was stable at room temperature for the duration of the study as the concentration of compound at the end of the subacute study was the same as the concentration at the start of the study.

b. The Stage-Wise Probit Procedure.

(1) The results of the Stage-Wise Probit Procedure are presented in Appendix E. None of the animals showed any signs of distress during the 14-day period of observation. Even at the limit dose of 2000 mg/kg, TAG-MNT showed no indication of toxicity. Confidence intervals are not reported since a lethal dose was not achieved.

(2) All animals in both Stage 1 and Stage 2 survived the 14-day observation period and were then euthanized. Gross pathology observations in these animals were unremarkable.

c. 14-Day Oral Repeated Dose Toxicity Study.

(1) A summary of results and raw data for the 14-day oral repeated dose toxicity study are presented in Appendices F-M.

(2) Clinical signs of toxicity were observed in the 2000 mg/kg-day dose group. These signs included, rough coat, piloerection, and lethargy at the end of the first week of dosing progressing to stained hair coat, a hunched or crouched gait, forelimb impairment, accompanied with significant weight loss. Five of the rats from this limit dose group lost greater than 20 percent of their starting body weight, and were removed from the study and euthanized beginning on day 8 up to day 13. Incidence of clinical signs are documented in the raw data.

(3) The net body weight change of the animals increased similarly with time for all dose groups except for the two highest dose groups, 1000 mg/kg and 2000 mg/kg. Weight gain was reduced in these two groups (see Appendices F and G). However, statistical significance was observed between treated and control animals only at Day 13 in the 2000 mg/kg group. At this time, five animals had already been removed from the study due to weight loss greater than 20 percent of starting body weight. The net food consumption during the first week was significantly reduced in the 1000 mg/kg and 2000 mg/kg groups (Appendix H).

(4) Differences were observed between the dose groups and the control group in mean organ to body weight ratios for the kidneys, liver, adrenal glands, and spleen. The 500-, 1000-, and 2000-mg/kg-day dose groups had elevated kidney and liver to body weight ratios when compared to methylcellulose control animals. The adrenals and spleen had decreased body weight ratios when compared to methylcellulose control animals. Differences between the treated and control groups in mean organ to brain weight ratios were also observed for the adrenal glands, spleen, kidneys and liver, at the highest two dose groups (Appendix K).

(5) Analysis of the clinical chemistry results revealed statistical significance for the ALB, ALT, BUN, CREA, GLU, and PHOS analytes when compared to the vehicle control group. The ALT, ALB and GLU were significantly decreased from control values at the 500-, 1000-, and 2000-mg/kg dose groups whereas BUN, CREA and Phos were significantly increased (Appendix I).

(6) Statistical analysis of the hematology results revealed significant differences for the absolute number of WBC, NEU, BASO, EOS and LYM when compared to the

control group; however there was no difference in the percentage of these cells in the total population. On the other hand, there was no decrease in the absolute number of monocytes in the WBC population; thus, the percentage MONOs significantly increased. There was no change in number of RBCs, hematocrit, or other hematologic parameters (Appendix J).

d. Histopathology was performed on selected liver and kidney tissues from all dose groups. No adverse events were observed in kidney. Dose-related adverse events were observed in the liver tissues. Toxicologically-relevant lesions included: single-cell necrosis (apoptotic), macrocytic and microcytic cytoplasmic vacuolization of hepatocytes, acute inflammation, hepatocyte degeneration, centrilobular and periportal hepatocyte hypertrophy, megakaryocytic (enlarged hepatocytes with enlarged nuclei) hepatocytes, increased mitoses (presence of mitotic hepatocytes), lymphocytic infiltration, and bile duct hyperplasia. Two control animals had minimal single-cell necrosis, but there was a clear dose relationship with increasing incidence and severity at the three highest doses. Trace vacuolar change was noted in three control animals, but there was a clear dose relationship with increasing incidence and severity at 250 mg/kg and above. Acute inflammation was noted in one animal at 500 mg/kg (mild) and two animals at 2000 mg/kg (mild to moderate), but not in the control group, and was most likely test article-related. Mild to moderate hepatocyte degeneration was present in one animal each of the two highest-dose groups, and was considered to be test article-related. Centrilobular and periportal hypertrophy were also dose-related, with the incidence and severity increasing with increasing dose (periportal hypertrophy was present only at the highest dose). Megakaryocytic hepatocytes were noted primarily at the highest dose, although minimal lesions were noted in one animal each at 250 and 500 m/kg. In addition, minimally increased hepatocyte mitotic activity was noted in one animal at 1000 mg/kg. Granulomatous inflammation or infiltration present in the control and treated groups was an incidental finding not considered to be related to treatment. The complete histopathology report is provided as Appendix M.

8. DISCUSSION.

a. The administration of TAG-MNT to female Sprague-Dawley rats did not cause acute toxicity up to the limit dose of 2000 mg/kg. However, daily administration for 14-days resulted in a dose-related toxicity primarily to the liver causing hepatocyte hypertrophy, inflammation, increased liver weight and weight loss; dosing with 2000 mg/kg-day resulted in 50 percent of the animals becoming moribund due to severe weight loss and removal from the study. Histopathologic scoring of liver adverse events established a NOAEL of 125 mg/kg-day.

b. The statistically significant increases in BUN and CREA in the high dose groups are consistent with dysfunction of the kidneys. However this was not supported by evidence of histopathology in the kidney tissues examined. The significant decrease in

ALT is counterintuitive as a marker of liver dysfunction (i.e., liver dysfunction is traditionally associated with an increase in plasma ALT).

c. There was a statistically significant reduction in white blood cells at TAG-MNT doses of 500 mg/kg and above. This included a general decrease in number of all subpopulations of WBCs, particularly neutrophils and lymphocytes, except for monocytes. The significance of this WBC toxicity and the apparent sparing of monocytes is not known.

d. The apparent dose-relationship of lymphocytic infiltration and bile duct hyperplasia in the liver histopathology is problematic. Lymphocytic infiltration was noted in the control group and at the three lowest doses of TAG-MNT but with decreasing incidence and severity in the higher-dose groups. Bile duct hyperplasia was present in the control group and the three lowest-dose groups but not at all at the higher doses. It is, therefore, questionable whether these lesions are attributable to the test article although they are often associated with the other liver lesions seen in this study.

e. The current USEPA regulatory values for RDX are based on reported chronic toxicities in rodents: a non-cancer RfD of 3 µg/kg-day based on prostatic inflammation in rodents (Levine et al., 1983), and a cancer slope factor of 0.11 mg/kg-day based on hepatocellular carcinomas in only female mice (Lish et al., 1984b; McLellan et al., 1992; Parker et al., 2006). However, the primary toxicity of RDX is its induction of epileptiform seizure and death following oral ingestion (Stone et al., 1969; Burdette et al., 1988; Kasuske et al., 2009). The mechanism of seizure induction has recently been shown to involve blockage of chloride flux through the GABAa receptor ligand-gated channel as a result of RDX binding to the convulsant site of the receptor (Williams et al., 2010).

f. TAG-MNT did not have affinity for the GABAa receptor when tested in an *in vitro* binding assay (data not shown). This result predicted that TAG-MNT would not induce convulsions when administered orally to female rats. In fact, oral administration of TAG-MNT did not cause any sign of acute toxicity up to the limit dose of 2000 mg/kg. The LD_{50} of RDX in rat is 60 mg/kg (Crouse et al., 2006). Thus, the acute oral toxicity of TAG-MNT is orders of magnitude less than RDX.

g. The 14-day NOAEL of RDX in rat is 15 mg/kg for which the adverse event is seizure and death. Daily administration of TAG-MNT for 14-days resulted in a dose-related toxicity primarily to the liver causing hepatocyte hypertrophy, inflammation, increased liver weight and severe weight loss and morbidity. The LOAEL of TAG-MNT based on increased kidney and liver weight ratios is 500 mg/kg-day. Sub-chronic, 90-day oral toxicity of TAG-MNT is speculated to be more severe with the adverse event being liver toxicity rather than seizure.

9. CONCLUSIONS.

a. TAG-MNT is not acutely toxic at the limit dose, but as early as 14-days of daily oral exposure causes liver toxicity, weight loss, and morbidity particularly at the highest dose. The primary target organ is the liver; TAG-MNT causes centrilobular hypertrophy, and increased liver weight. This is correlated with decreased food consumption in the higher dose groups, severe body weight loss and morbidity.

b. Measured signs of toxicity in the 14-day study at dosages of 500 mg/kg-day and above included decreased food consumption as well as alterations in the kidney and liver weight ratios when compared to controls. Histopathologic evidence of adverse events were observed down to 250 mg/kg-day.

c. The lowest-observed-effect-level (LOAEL) from oral exposure to TAG-MNT for 14-days, as determined from this study, is 500 mg/kg-day based on significant adverse event of increased kidney and liver weight ratios and adverse histological alterations in the liver. The no-observed-adverse-effect-level is therefore determined to be 250 mg/kg-d where only mild adaptive hepatic vacuolization was observed in a subset of exposed individuals.

APPENDIX A
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APPENDIX B

For: Toxicology Study No. 87-XE-0BME-09, Protocol No. 0BME-30-09-03-01 entitled "Effects of Acute and Sub-Acute (14 Day) Oral Exposure of Triaminoguanidinium-1-methyl-5 nitriminotetrazolate (TAG-MNT) in Female Rats" the following critical phases were inspected/audited by the Quality Systems Office (QSO):

PRE IN-LIFE PHASE OF THE STUDY

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
GLP Protocol Review	02/24/2009	02/24/2009

IN-LIFE LD50 DETERMINATION USING THE SSWP METHOD PHASE OF THE STUDY

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Test Article - Facilities, Control and Handling	10/09/2009	10/19/2009
Test System – Facilities and Identification	10/09/2009	10/14/2009
Compliance with DTOX SOPs	10/09/2009	10/19/2009
Maintenance and Calibration of Equipment	10/16/2009	10/19/2009
Compliance with DTOX Protocols	10/16/2009	10/19/2009
Test System - Food and Water Supply	10/16/2009	10/19/2009
Test Systems - Husbandry and Observations	10/16/2009	10/19/2009
Pre-Procedural Provisions	10/27/2009	10/29/2009
Euthanasia procedures	10/27/2009	10/29/2009
Gross Necropsy - Raw Data and Records Review	10/27/2009	10/29/2009

IN-LIFE 14 DAY REPEATED DOSE PHASE OF THE STUDY

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Test System - Microchipping	11/20/2009	11/25/2009
Test System – Pre-Study Weights	11/20/2009	11/25/2009
LabCat Procedures	11/20/2009	11/25/2009
Test System - Facilities, Receipt and Identification	12/02/2009	12/03/2009

Note: All findings were made known to the Study Director and the Program Manager at the time of the audit/inspection. If there were no findings during the inspection, the inspection was reported to Management and the Study Director on the date shown in the table.

APPENDIX B

For: Toxicology Study No. 87-XE-0BME-09, Protocol No. 0BME-30-09-03-01 entitled "Effects of Acute and Sub-Acute (14 Day) Oral Exposure of Triaminoguanidinium-1-methyl-5 nitriminotetrazolate (TAG-MNT) in Female Rats" the following critical phases were inspected/audited by the Quality Systems Office (QSO):

IN LIFE 14 DAY REPEATED DOSE PHASE OF THE STUDY (continued)

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Test Systems - Husbandry and Observations	12/02/2009	12/03/2009
Test Article - Control and Dosing	12/02/2009	12/03/2009
Compliance with DTOX Protocols	12/03/2009	12/10/2009
Raw Data Documentation Procedures	12/10/2009	12/22/2009
Necropsy-General Requirements and Procedures	12/10/2009	12/15/2009
Organ and Tissue Processing and Preservation	12/10/2009	12/15/2009
Compliance with DTOX SOPs	12/10/2009	12/22/2009
Blood Collection for the Micronucleus Assay	12/10/2009	12/15/2009

POST IN-LIFE PHASE OF THE STUDY

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Reinspection of Corrective Action(s)	01/21/2010	01/22/10
Reinspection of Corrective Action(s)	01/21/2010	01/22/10
Final Study Report Review	05/27/2010	06/04/10
Study Raw Data Review	05/27/2010	06/04/10

Note: All findings were made known to the Study Director and the Program Manager at the time of the audit/inspection. If there were no findings during the inspection, the inspection was reported to Management and the Study Director on the date shown in the table.

Michael P. Kefauver
GLP Assessor, QSO

08/10/2010
Date

Gene Sinar
Team Leader, QSO

8/10/2010
Date

APPENDIX C
ARCHIVES AND STUDY PERSONNEL

C-1. ARCHIVES.

a. All raw data, documentation, records, protocol, and a copy of the final report generated as a result of this study will be archived in the storage facilities of the Toxicology Directorate, USAPHC (Prov), for a minimum of five (5) years following submission of the final report to the Sponsor. If the report is used to support a regulatory action, it shall, along with all supporting data, be retained indefinitely.

b. Records on animal receipt, diet, and facility environmental parameters will be archived by the Veterinary Medical Division, Toxicology Directorate, for a minimum of five (5) years following submission of the final report to the Sponsor. If the report is used to support a regulatory action, it shall, along with all supporting data, be retained indefinitely.

c. The present study used the laboratory project number: 87-XE-0BMEA-10, protocol number 0BME-30-09-03-01 for all filings.

d. The protocol, raw data, summary data, and the final report pertaining to this study will be physically maintained within Building E-2100, USAPHC (Prov). These data may be scanned to a computer disk. Scanned study files will be stored electronically in Room 3010, Building E-2100, USAPHC (Prov), APG, MD, 21010.

e. Archived SOPs and maintenance and calibration logbooks may be found in Room 1026, Building E-2100, USAPHC (Prov), APG, MD, 21010.

f. Records on animal receipt, diet, and environmental parameters are maintained in Room 3014, Building E-2100, USAPHC (Prov), APG, MD, 21010.

g. Wet tissues are stored in cage 12 of Building 1958, USAPHC (Prov), APG, MD, 21010.

h. Histology slides, paraffin blocks, and hematology slides are stored in the basement of Building 1570, USAPHC (Prov), APG, MD, 21010.

i. Archivist: Kristin Newkirk.

C-2. PERSONNEL.

a. Management: Cindy A. Landgren, LTC, Director, Toxicology; Mark Johnson, Manager, Health Effects Research Program (HERP)

b. Study Director: Larry Williams, Biologist, HERP.

c. Veterinary Support, Necropsies, and Animal Care: Anne MacLarty, DVM, MAJ, VC, Toxicity Evaluation Program (TEP), Patricia Beal, TEP, and Wilfred McCain, Ph.D., Toxicologist, TEP.

d. Hematology, Clinical Chemistry: Matthew Bazar, Biologist, TEP.

e. Computer Software Support: Martha Thompson, Data Acquisition Specialist, TEP.

f. Animal Care: Robert Sunderland, Theresa Hanna, Jason Williams, Rebecca Kilby, TEP.

g. In-Life Support: Mark Way, Biologist, TEP; Matthew Bazar, Biologist, TEP; John Houpt, Biologist, TEP; Patricia Beall, Biologist, TEP.

h. Pathology Laboratory Coordinator: Patricia Beall, Biologist, TEP.

APPENDIX D
ANALYTICAL DATA
SUMMARY OF 14-DAY ANALYTICAL RESULTS
REPORTED CONCENTRATIONS (mg/mL)

Protocol No. 0BME-30-09-03-01
Oral Toxicity of TAG-MNT in Female Rats

Dosing Solution Homogeneity Test 11-9-09

Location Sampled	Analytical (mg/mL)	Nominal (mg/mL)
Top	160	200
Middle	150	200
Bottom	150	200

14-Day Dosing Solution Three Weeks Stability at 4°C

Date Sampled	Analytical (mg/mL)	Nominal (mg/mL)
10-26-09	6.6	6.5
11-02-09	7.5	6.5
11-09-09	7.7	6.5
11-16-09	7.5	6.5

Results of homogeneity study indicated that method of preparation over estimated actual concentration of compound. Thus, stock solutions were prepared deliberately 20 percent more concentrated than desired: 240 mg/mL, 60 mg/mL, and 15 mg/mL.

Dosing Solution	Analytical (mg/mL)	Nominal (mg/mL)
11-20-09		
High	245	240
Middle	74	60
Low	18	15

14-Day Dosing Solution Three Weeks Stability at Room Temp

Dosing Solution	11-20-09	12-09-09
High	245	238
Middle	74	74
Low	18	17

Date Sampled	Analytical (mg/mL)	Nominal (mg/mL)
11-20-09	245	245
11-24-09	245	245
12-09-09	238	245
11-20-09	190	200
11-25-09	156	200
12-09-09	211	200

APPENDIX E
SEQUENTIAL STAGE-WISE PROBIT (SSWP): ORAL, RAT

Table E-1
Protocol No. 0BME-30-09-03-01
Acute Oral Toxicity of TAG-MNT in Female Rats

Study No. 87-XE-0BMEA-10, Protocol No. 0BME-30-09-03-01 SOP No. 17.08
Chemical Substance: Triaminoguanidinium-1-methyl-5-nitriminotetrazolate (TAG-MNT)
Route: Oral Species: Sprague-Dawley Rat Sex: Female
Diluent: Corn Oil or 1 percent methylcellulose, 0.2 percent Tween 80, in tap water

Animal No.	Diluent	Dosing Stage	Weight Kg	Nominal Dose mg/kg	Volume mL	Exposure Day Signs	Exposure Day Morbidity/Mortality
09-825	Corn Oil	1	0.176	180	1.5	N	No
09-826	Corn Oil	1	0.171	270	1.5	N	No
09-827	Corn Oil	1	0.164	400	1.5	N	No
09-828	Corn Oil	1	0.186	600	1.5	N	No
09-829	Corn Oil	1	0.177	900	1.5	N	No
09-830	Corn Oil	1	0.172	1350	1.5	N	No
09-831	Corn Oil	1	0.176	2000	1.5	N	No
09-832	Corn Oil	2	0.186	2000	1.5	N	No
09-833	Corn Oil	2	0.195	2000	1.5	N	No
09-834	Corn Oil	2	0.201	2000	1.5	N	No
09-835	Methyl Cellulose	2	0.196	2000	1.5	N	No
09-836	Methyl Cellulose	2	0.211	2000	1.5	N	No
09-837	Methyl Cellulose	2	0.184	2000	1.5	N	No

*Signs: N–Normal S1-elevated respiration S2-squinting S3-prostrate S4 –found dead S5-labored breathing

Study Conclusions – TAG-MNT is not acutely toxic

Table E-2
Protocol No. 0BME-30-09-03-01
Subacute Oral Toxicity of TAG-MNT in Female Rats

Acute SSWP Individual Body Weights (grams)

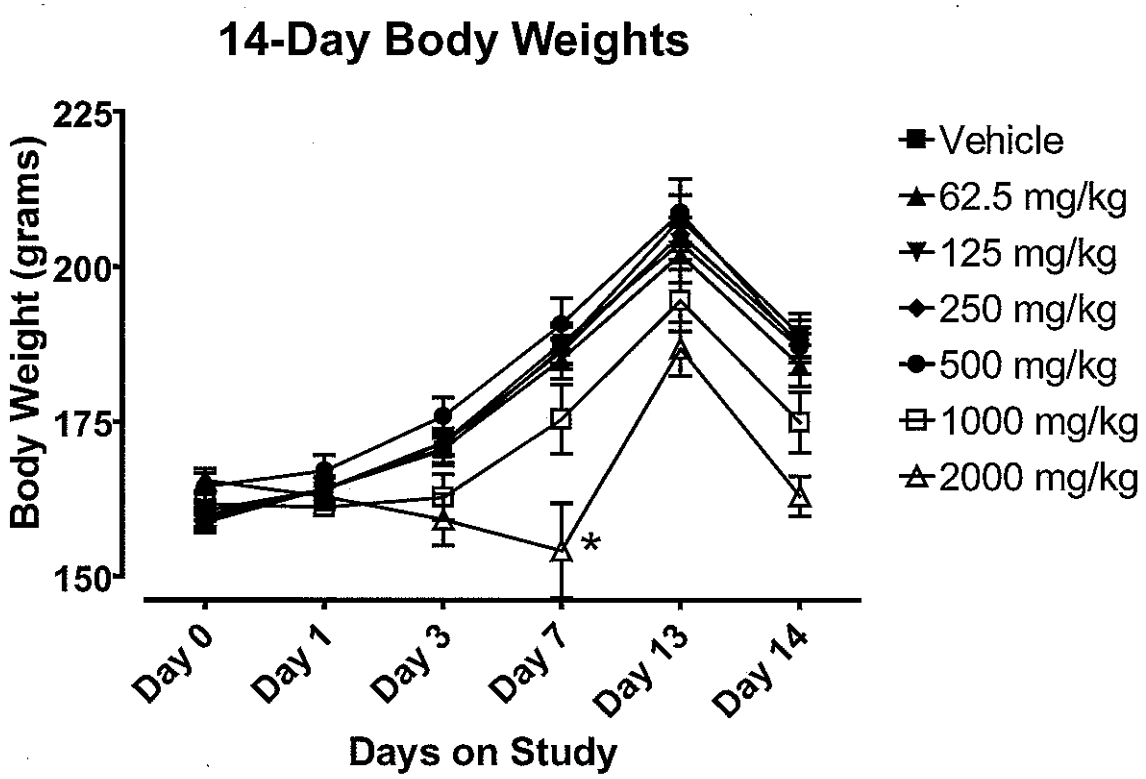
		Individual Body Weights (grams)				Individual Body Weight Changes (grams)			
			Fasted						
Phase 1 Dose mg/kg	**Animal ID**	**Pre Wt**	**Day 0**	**Day 5**	**Day 14**	**Day Pre-0**	**Day o-5**	**Day 5-14**	**Net Change**
180	0825-09	193	176	213	241	-17	37	28	48
270	0826-09	186	171	209	238	-15	38	29	52
400	0827-09	179	164	200	219	-15	36	19	40
600	0828-09	202	187	226	259	-15	39	33	57
900	0829-09	190	178	210	238	-12	32	28	48
1350	0830-09	182	173	200	223	-9	27	23	41
2000	0831-09	193	176	204	236	-17	28	32	43
Mean		**189**	**175**	**209**	**236**	**-14**	**34**	**27**	**47**
SD		**7.740**	**7.024**	**9.063**	**13.035**	**2.870**	**4.880**	**4.928**	**6.164**

Phase 2	Animal ID	Day 0	Day 9	Day 14	Day 0-9	Day 9-14	Net Change
2000 mg/kg Corn Oil	0832-09	187	217	228	30	11	41
2000 mg/kg Corn Oil	0833-09	196	242	261	46	19	65
2000 mg/kg Corn Oil	0834-09	201	238	257	37	19	56
2000 mg/kg Methyl Cellulose	0835-09	197	234	251	37	17	54
2000 mg/kg Methyl Cellulose	0836-09	211	254	267	43	13	56
2000 mg/kg Methyl Cellulose	0837-09	184	223	231	39	8	47
Mean		**196**	**235**	**249**	**39**	**15**	**53**
SD		**9.757**	**13.322**	**16.130**	**5.538**	**4.550**	**8.280**

APPENDIX F

SUMMARY OF 14-DAY BODY WEIGHTS
AND INDIVIDUAL DATA

Protocol No. 0BME-30-09-03-01
Subacute Oral Toxicity of TAG-MNT in Female Rats



Note: Rats were fasted overnight prior to necropsy on Day 14. Five animals in the 2000-mg/kg dosing group were removed from the study between Day 7 and Day 13 due weight loss exceeding 20 percent of initial body weight. *$p \leq 0.05$

Figure F-1. 14-Day Body Weights and Individual Data

Table F-1
Protocol No. 08UG-70-IV09-06-01
Subacute Oral Toxicity of TAG-MNT in Female Rats

Summary of 14-Day Body Weights (grams)

		Vehicle Control	TAG-MNT (mg/kg)					
Period		0	62.5	125	250	500	1000	2000
Day 0	**Mean**	158.8	159.3	160.6	159.9	164.5	161.7	165.5
	S.D.	5.6	5.5	5.1	6.0	7.1	5.6	6.0
	N	10	10	10	10	10	10	10
Day 1	**Mean**	164.2	164.1	164.1	164.1	167.1	161.2	162.9
	S.D.	6.3	5.8	6.7	6.4	7.9	4.0	6.6
	N	10	10	10	10	10	10	10
Day 3	**Mean**	170.4	170.7	171.7	171.5	175.9	162.8	159.2
	S.D.	6.4	8.8	7.0	5.9	9.5	11.7	13.2
	N	10	10	10	10	10	10	10
Day 7	**Mean**	186.9	185.1	187.7	186.4	190.8	175.4	154.2
	S.D.	10.9	10.0	10.3	6.8	13.1	17.6	24.2
	N	10	10	10	10	10	10	10
Day 13	**Mean**	207.8	202	203.9	205.3	208.8	194.6	186.8
	S.D.	12.1	14.1	8.5	8.6	17.0	15.7	13.755
	N	10	10	10	10	10	10	5
Day 14	**Mean**	189	184.1	186.9	187.8	187.1	174.9	163
	S.D.	11.0	10.5	7.4	7.8	13.8	15.5	10.1
	N	10	10	10	10	10	10	5

Table F-2
Protocol No. 08UG-70-IV09-06-01
Subacute Oral Toxicity of TAG-MNT in Female Rats

14-Day Individual Body Weights (grams)

	Animal ID	Day 0	Day 1	Day 3	Day 7	Day 13	Day 14
Vehicle	0009-10F	153	160	163	171	205	185
Control	0030-10F	160	165	170	183	204	183
	0035-10F	159	161	172	187	215	196
	0038-10F	172	180	187	210	234	208
	0044-10F	156	160	168	182	200	185
	0061-10F	161	165	170	183	194	174
	0069-10F	156	158	170	186	200	180
	0078-10F	156	163	166	183	201	185
	0085-10F	153	162	170	183	204	188
	0087-10F	162	168	168	201	221	206
Mean		**159**	**164**	**170**	**187**	**208**	**189**
SD		**5.594**	**6.286**	**6.363**	**10.908**	**12.054**	**11.005**

	Animal ID	Day 0	Day 1	Day 3	Day 7	Day 13	Day 14
62.5 mg/kg	0013-10F	159	163	169	175	186	171
	0015-10F	161	162	174	192	217	194
	0016-10F	152	162	158	176	188	171
	0031-10F	165	165	171	184	191	179
	0040-10F	149	152	156	171	189	177
	0053-10F	160	170	177	190	206	188
	0066-10F	163	167	172	189	202	183
	0067-10F	159	163	172	180	204	187
	0077-10F	167	174	187	205	230	205
	0081-10F	158	163	171	189	207	186
Mean		**159**	**164**	**171**	**185**	**202**	**184**
SD		**5.478**	**5.782**	**8.820**	**10.049**	**14.126**	**10.450**

	Animal ID	Day 0	Day 1	Day 3	Day 7	Day 13	Day 14
125 mg/kg	0005-10F	159	164	177	193	211	192
	0029-10F	166	173	177	195	201	189
	0032-10F	159	160	167	178	198	183
	0034-10F	158	159	164	176	191	180
	0037-10F	158	157	167	176	195	177
	0043-10F	157	162	168	191	205	184
	0072-10F	156	160	166	186	206	187
	0074-10F	156	159	167	179	202	181
	0079-10F	167	172	182	197	220	200
	0090-10F	170	175	182	206	210	196
Mean		**161**	**164**	**172**	**188**	**204**	**187**
SD		**5.082**	**6.674**	**6.993**	**10.328**	**8.465**	**7.370**

Table F-2
Protocol No. 08UG-70-IV09-06-01
Subacute Oral Toxicity of TAG-MNT in Female Rats

14-Day Individual Body Weights (grams)

Table F-2 (continued)

	Animal ID	Day 0	Day 1	Day 3	Day 7	Day 13	Day 14
250 mg/kg	0006-10F	162	167	176	187	200	185
	0024-10F	163	172	177	198	207	190
	0025-10F	169	174	179	193	211	193
	0036-10F	155	161	166	180	204	189
	0042-10F	167	166	175	193	218	198
	0055-10F	149	152	163	176	194	178
	0060-10F	158	161	166	181	192	180
	0070-10F	160	160	173	188	217	201
	0075-10F	161	167	175	183	205	183
	0089-10F	155	161	165	185	205	181
Mean		160	164	172	186	205	188
SD		5.953	6.437	5.855	6.802	8.616	7.786

	Animal ID	Day 0	Day 1	Day 3	Day 7	Day 13	Day 14
500 mg/kg	0003-10F	179	180	187	202	220	194
	0004-10F	160	161	165	179	200	176
	0020-10F	160	160	171	180	193	172
	0021-10F	172	179	187	213	232	204
	0033-10F	158	163	171	186	202	184
	0052-10F	164	163	171	181	181	166
	0063-10F	166	167	176	190	206	190
	0065-10F	169	175	191	208	235	210
	0076-10F	160	164	176	194	216	192
	0082-10F	157	159	164	175	203	183
Mean		165	167	176	191	209	187
SD		7.059	7.937	9.469	13.122	16.963	13.796

	Animal ID	Day 0	Day 1	Day 3	Day 7	Day 13	Day 14
1000 mg/kg	0001-10F	167	163	153	174	180	155
	0007-10F	165	162	170	181	202	179
	0011-10F	160	158	164	145	194	169
	0023-10F	158	159	136	146	173	147
	0045-10F	172	167	174	193	213	189
	0048-10F	155	160	160	177	192	175
	0050-10F	155	155	160	171	184	168
	0056-10F	165	161	166	187	208	189
	0068-10F	163	168	176	196	220	196
	0084-10F	157	159	169	184	180	182
Mean		162	161	163	175	195	175
SD		5.638	3.994	11.698	17.595	15.742	15.517

Table F-2
Protocol No. 08UG-70-IV09-06-01
Subacute Oral Toxicity of TAG-MNT in Female Rats

14-Day Individual Body Weights (grams)

Table F-2 (continued)

	Animal ID	Day 0	Day 1	Day 3	Day 7	Day 13	Day 14
2000 mg/kg	0008-10F	167	158	146	126	f	
	0017-10F	168	167	149	119	f	
	0046-10F	171	164	163	164	180	156
	0047-10F	150	154	157	169	192	167
	0051-10F	168	163	163	172	166	149
	0059-10F	164	152	134	119	f	
	0071-10F	169	172	171	184	198	171
	0073-10F	165	163	162	157	f	
	0083-10F	163	165	168	176	198	172
	0088-10F	170	171	179	156	f	
Mean		**166**	**163**	**159**	**154**	**187**	**163**
SD		**6.023**	**6.607**	**13.181**	**24.220**	**13.755**	**10.075**

f = Animal removed from study due to severe weight loss

APPENDIX G

SUMMARY OF 14-DAY BODY WEIGHT CHANGES
AND INDIVIDUAL DATA

Protocol No. 0BME-30-09-03-01
Subacute Oral Toxicity of TAG-MNT in Female Rats

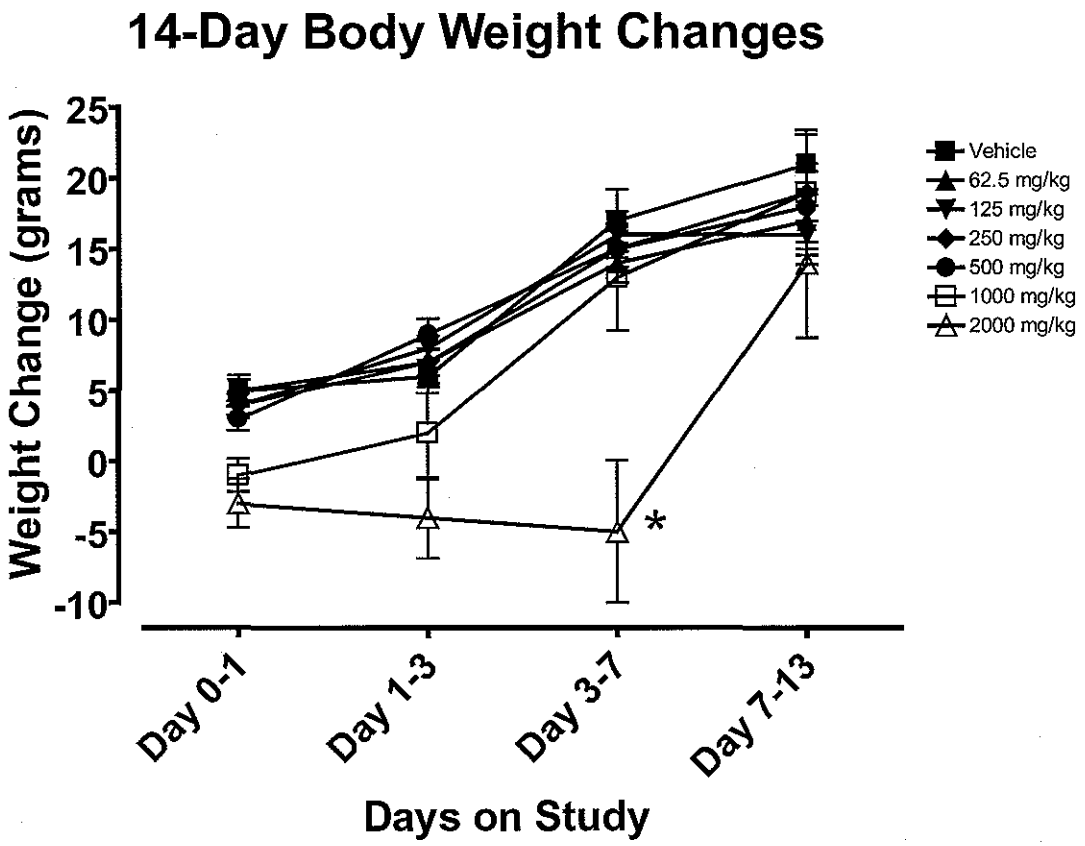


Figure G-1. Summary of 14-Day Body Weight Changes and Individual Data

Note: Rats were fasted overnight prior to necropsy on Day 14. Five animals in the 2000 mg/kg dosing group were removed from the study between Day 7 and Day 13 due weight loss exceeding 20 percent of initial body weight. *$p \leq 0.05$

Table G-1
Protocol No. 08UG-70-IV09-06-01
Subacute Oral Toxicity of TAG-MNT in Female Rats

Summary of 14-Day Body Weight Changes (grams)

		Vehicle Control	TAG-MNT (mg/kg)					
Period		0	62.5	125	250	500	1000	2000
Day 0-1	**Mean**	5	5	4	4	3	-1	-3
	S.D.	2.4	3.4	2.5	3.0	2.7	3.7	5.4
	N	10	10	10	10	10	10	10
Day 1-3	**Mean**	6	7	8	7	9	2	-4
	S.D.	3.7	4.7	2.7	3.1	3.5	10.4	9.1
	N	10	10	10	10	10	10	10
Day 3-7	**Mean**	17	14	16	15	15	13	-5
	S.D.	6.9	4.4	5.2	4.0	4.9	11.9	15.9
	N	10	10	10	10	10	10	10
Day 7-13	**Mean**	21	17	16	19	18	19	14
	S.D.	6.6	6.3	6.6	6.4	7.9	13.9	11.7
	N	10	10	10	10	10	10	5
Net	**Mean**	30	25	26	28	23	13	-1
	S.D.	8.7	8.3	4.7	6.1	11.4	14.7	15.4
	N	10	10	10	10	10	10	5

Table G-2
Protocol No. 08UG-70-IV09-06-01
Subacute Oral Toxicity of TAG-MNT in Female Rats

14-Day Individual Body Weight Changes (grams)

	Animal ID	Day 0-1	Day 1-3	Day 3-7	Day 7-13	Day 13-14	Net Change
Vehicle	0009-10F	7	3	8	34	-20	32
Control	0030-10F	5	5	13	21	-21	23
	0035-10F	2	11	15	28	-19	37
	0038-10F	8	7	23	24	-26	36
	0044-10F	4	8	14	18	-15	29
	0061-10F	4	5	13	11	-20	13
	0069-10F	2	12	16	14	-20	24
	0078-10F	7	3	17	18	-16	29
	0085-10F	9	8	13	21	-16	35
	0087-10F	6	0	33	20	-15	44
Mean		**5**	**6**	**17**	**21**	**-19**	**30**
SD		**2.413**	**3.736**	**6.932**	**6.624**	**3.425**	**8.728**

	Animal ID	Day 0-1	Day 1-3	Day 3-7	Day 7-13	Day 13-14	Net Change
62.5 mg/kg	0013-10F	4	6	6	11	-15	12
	0015-10F	1	12	18	25	-23	33
	0016-10F	10	-4	18	12	-17	19
	0031-10F	0	6	13	7	-12	14
	0040-10F	3	4	15	18	-12	28
	0053-10F	10	7	13	16	-18	28
	0066-10F	4	5	17	13	-19	20
	0067-10F	4	9	8	24	-17	28
	0077-10F	7	13	18	25	-25	38
	0081-10F	5	8	18	18	-21	28
Mean		**5**	**7**	**14**	**17**	**-18**	**25**
SD		**3.360**	**4.719**	**4.402**	**6.297**	**4.306**	**8.297**

	Animal ID	Day 0-1	Day 1-3	Day 3-7	Day 7-13	Day 13-14	Net Change
125 mg/kg	0005-10F	5	13	16	18	-19	33
	0029-10F	7	4	18	6	-12	23
	0032-10F	1	7	11	20	-15	24
	0034-10F	1	5	12	15	-11	22
	0037-10F	-1	10	9	19	-18	19
	0043-10F	5	6	23	14	-21	27
	0072-10F	4	6	20	20	-19	31
	0074-10F	3	8	12	23	-21	25
	0079-10F	5	10	15	23	-20	33
	0090-10F	5	7	24	4	-14	26
Mean		**4**	**8**	**16**	**16**	**-17**	**26**
SD		**2.461**	**2.716**	**5.164**	**6.596**	**3.712**	**4.739**

Table G-2
Protocol No. 08UG-70-IV09-06-01
Subacute Oral Toxicity of TAG-MNT in Female Rats

14-Day Individual Body Weight Changes (grams)

Table G-2 (continued)

	Animal ID	Day 0-1	Day 1-3	Day 3-7	Day 7-13	Day 13-14	Net Change
250 mg/kg	0006-10F	5	9	11	13	-15	23
	0024-10F	9	5	21	9	-17	27
	0025-10F	5	5	14	18	-18	24
	0036-10F	6	5	14	24	-15	34
	0042-10F	-1	9	18	25	-20	31
	0055-10F	3	11	13	18	-16	29
	0060-10F	3	5	15	11	-12	22
	0070-10F	0	13	15	29	-16	41
	0075-10F	6	8	8	22	-22	22
	0089-10F	6	4	20	20	-24	26
Mean		**4**	**7**	**15**	**19**	**-18**	**28**
SD		**3.011**	**3.062**	**3.957**	**6.437**	**3.598**	**6.082**

	Animal ID	Day 0-1	Day 1-3	Day 3-7	Day 7-13	Day 13-14	Net Change
500 mg/kg	0003-10F	1	7	15	18	-26	15
	0004-10F	1	4	14	21	-24	16
	0020-10F	0	11	9	13	-21	12
	0021-10F	7	8	26	19	-28	32
	0033-10F	5	8	15	16	-18	26
	0052-10F	-1	8	10	0	-15	2
	0063-10F	1	9	14	16	-16	24
	0065-10F	6	16	17	27	-25	41
	0076-10F	4	12	18	22	-24	32
	0082-10F	2	5	11	28	-20	26
Mean		**3**	**9**	**15**	**18**	**-22**	**23**
SD		**2.716**	**3.490**	**4.864**	**7.916**	**4.398**	**11.443**

	Animal ID	Day 0-1	Day 1-3	Day 3-7	Day 7-13	Day 13-14	Net Change
1000 mg/kg	0001-10F	-4	-10	21	6	-25	-12
	0007-10F	-3	8	11	21	-23	14
	0011-10F	-2	6	-19	49	-25	9
	0023-10F	1	-23	10	27	-26	-11
	0045-10F	-5	7	19	20	-24	17
	0048-10F	5	0	17	15	-17	20
	0050-10F	0	5	11	13	-16	13
	0056-10F	-4	5	21	21	-19	24
	0068-10F	5	8	20	24	-24	33
	0084-10F	2	10	15	-4	2	25
Mean		**-1**	**2**	**13**	**19**	**-20**	**13**
SD		**3.689**	**10.362**	**11.890**	**13.935**	**8.407**	**14.711**

Table G-2
Protocol No. 08UG-70-IV09-06-01
Subacute Oral Toxicity of TAG-MNT in Female Rats

14-Day Individual Body Weight Changes (grams)

Table G-2 (continued)

	Animal ID	Day 0-1	Day 1-3	Day 3-7	Day 7-13	Day 13-14	Net Change
2000 mg/kg	0008-10F	-9	-12	-20			
	0017-10F	-1	-18	-30			
	0046-10F	-7	-1	1	16	-24	-15
	0047-10F	4	3	12	23	-25	17
	0051-10F	-5	0	9	-6	-17	-19
	0059-10F	-12	-18	-15			
	0071-10F	3	-1	13	14	-27	2
	0073-10F	-2	-1	-5			
	0083-10F	2	3	8	22	-26	9
	0088-10F	1	8	-23			
Mean		**-3**	**-4**	**-5**	**14**	**-24**	**-1**
SD		**5.441**	**9.068**	**15.944**	**11.713**	**3.962**	**15.434**

Table G-2
Protocol No. 08UG-70-IV09-06-01
Subacute Oral Toxicity of TAG-MNT in Female Rats

14-Day Individual Body Weight Changes (grams)

	Animal ID	Day 0-1	Day 1-3	Day 3-7	Day 7-13	Day 13-14	Net Change
Vehicle	0009-10F	7	3	8	34	-20	32
Control	0030-10F	5	5	13	21	-21	23
	0035-10F	2	11	15	28	-19	37
	0038-10F	8	7	23	24	-26	36
	0044-10F	4	8	14	18	-15	29
	0061-10F	4	5	13	11	-20	13
	0069-10F	2	12	16	14	-20	24
	0078-10F	7	3	17	18	-16	29
	0085-10F	9	8	13	21	-16	35
	0087-10F	6	0	33	20	-15	44
Mean		**5**	**6**	**17**	**21**	**-19**	**30**
SD		**2.413**	**3.736**	**6.932**	**6.624**	**3.425**	**8.728**

	Animal ID	Day 0-1	Day 1-3	Day 3-7	Day 7-13	Day 13-14	Net Change
62.5 mg/kg	0013-10F	4	6	6	11	-15	12
	0015-10F	1	12	18	25	-23	33
	0016-10F	10	-4	18	12	-17	19
	0031-10F	0	6	13	7	-12	14
	0040-10F	3	4	15	18	-12	28
	0053-10F	10	7	13	16	-18	28
	0066-10F	4	5	17	13	-19	20
	0067-10F	4	9	8	24	-17	28
	0077-10F	7	13	18	25	-25	38
	0081-10F	5	8	18	18	-21	28
Mean		**5**	**7**	**14**	**17**	**-18**	**25**
SD		**3.360**	**4.719**	**4.402**	**6.297**	**4.306**	**8.297**

	Animal ID	Day 0-1	Day 1-3	Day 3-7	Day 7-13	Day 13-14	Net Change
125 mg/kg	0005-10F	5	13	16	18	-19	33
	0029-10F	7	4	18	6	-12	23
	0032-10F	1	7	11	20	-15	24
	0034-10F	1	5	12	15	-11	22
	0037-10F	-1	10	9	19	-18	19
	0043-10F	5	6	23	14	-21	27
	0072-10F	4	6	20	20	-19	31
	0074-10F	3	8	12	23	-21	25
	0079-10F	5	10	15	23	-20	33
	0090-10F	5	7	24	4	-14	26
Mean		**4**	**8**	**16**	**16**	**-17**	**26**
SD		**2.461**	**2.716**	**5.164**	**6.596**	**3.712**	**4.739**

Table G-2
Protocol No. 08UG-70-IV09-06-01
Subacute Oral Toxicity of TAG-MNT in Female Rats

14-Day Individual Body Weight Changes (grams)

Table G-2 (continued)

	Animal ID	Day 0-1	Day 1-3	Day 3-7	Day 7-13	Day 13-14	Net Change
250 mg/kg	0006-10F	5	9	11	13	-15	23
	0024-10F	9	5	21	9	-17	27
	0025-10F	5	5	14	18	-18	24
	0036-10F	6	5	14	24	-15	34
	0042-10F	-1	9	18	25	-20	31
	0055-10F	3	11	13	18	-16	29
	0060-10F	3	5	15	11	-12	22
	0070-10F	0	13	15	29	-16	41
	0075-10F	6	8	8	22	-22	22
	0089-10F	6	4	20	20	-24	26
Mean		**4**	**7**	**15**	**19**	**-18**	**28**
SD		**3.011**	**3.062**	**3.957**	**6.437**	**3.598**	**6.082**

	Animal ID	Day 0-1	Day 1-3	Day 3-7	Day 7-13	Day 13-14	Net Change
500 mg/kg	0003-10F	1	7	15	18	-26	15
	0004-10F	1	4	14	21	-24	16
	0020-10F	0	11	9	13	-21	12
	0021-10F	7	8	26	19	-28	32
	0033-10F	5	8	15	16	-18	26
	0052-10F	-1	8	10	0	-15	2
	0063-10F	1	9	14	16	-16	24
	0065-10F	6	16	17	27	-25	41
	0076-10F	4	12	18	22	-24	32
	0082-10F	2	5	11	28	-20	26
Mean		**3**	**9**	**15**	**18**	**-22**	**23**
SD		**2.716**	**3.490**	**4.864**	**7.916**	**4.398**	**11.443**

	Animal ID	Day 0-1	Day 1-3	Day 3-7	Day 7-13	Day 13-14	Net Change
1000 mg/kg	0001-10F	-4	-10	21	6	-25	-12
	0007-10F	-3	8	11	21	-23	14
	0011-10F	-2	6	-19	49	-25	9
	0023-10F	1	-23	10	27	-26	-11
	0045-10F	-5	7	19	20	-24	17
	0048-10F	5	0	17	15	-17	20
	0050-10F	0	5	11	13	-16	13
	0056-10F	-4	5	21	21	-19	24
	0068-10F	5	8	20	24	-24	33
	0084-10F	2	10	15	-4	2	25
Mean		**-1**	**2**	**13**	**19**	**-20**	**13**
SD		**3.689**	**10.362**	**11.890**	**13.935**	**8.407**	**14.711**

Table G-2
Protocol No. 08UG-70-IV09-06-01
Subacute Oral Toxicity of TAG-MNT in Female Rats

14-Day Individual Body Weight Changes (grams)

Table G-2 (continued)

	Animal ID	Day 0-1	Day 1-3	Day 3-7	Day 7-13	Day 13-14	Net Change
2000 mg/kg	0008-10F	-9	-12	-20			
	0017-10F	-1	-18	-30			
	0046-10F	-7	-1	1	16	-24	-15
	0047-10F	4	3	12	23	-25	17
	0051-10F	-5	0	9	-6	-17	-19
	0059-10F	-12	-18	-15			
	0071-10F	3	-1	13	14	-27	2
	0073-10F	-2	-1	-5			
	0083-10F	2	3	8	22	-26	9
	0088-10F	1	8	-23			
Mean		**-3**	**-4**	**-5**	**14**	**-24**	**-1**
SD		**5.441**	**9.068**	**15.944**	**11.713**	**3.962**	**15.434**

APPENDIX H

SUMMARY OF 14-DAY FOOD CONSUMPTION
AND INDIVIDUAL DATA

Protocol No. 0BME-30-09-03-01
Subacute Oral Toxicity of TAG-MNT in Female Rats[1]

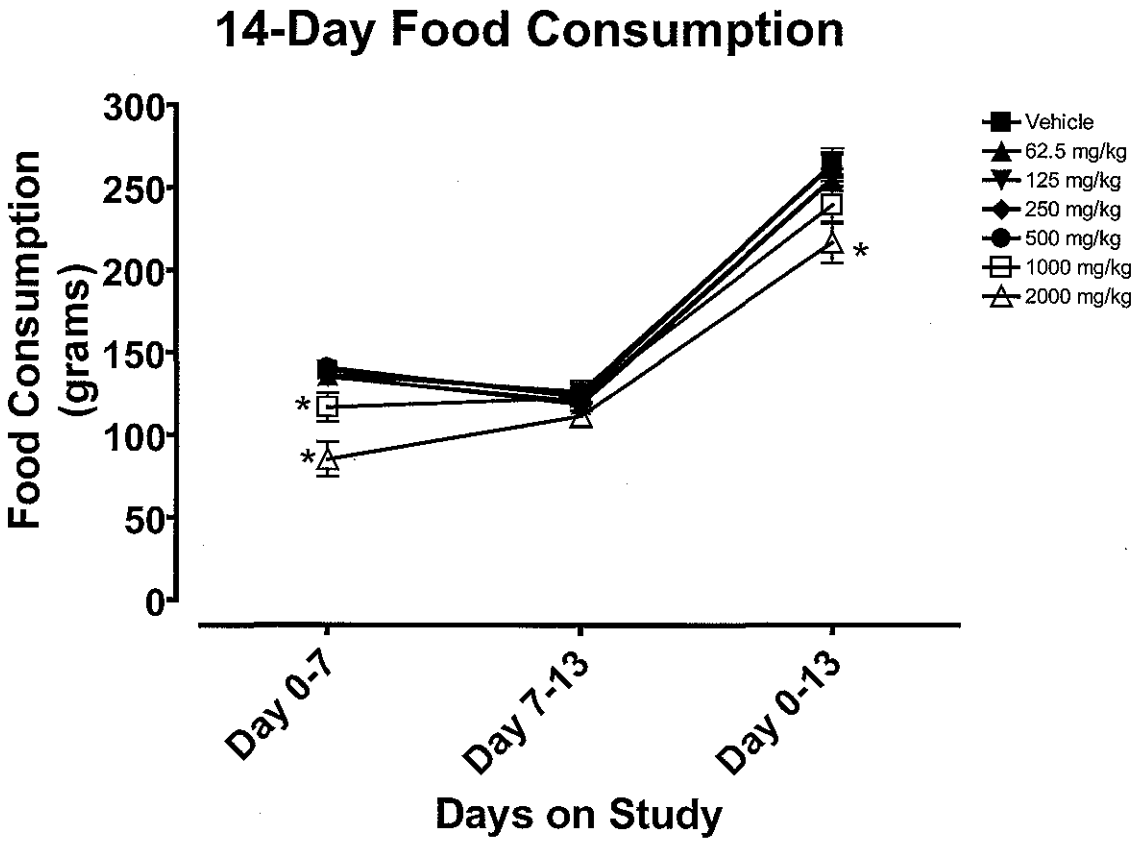


Figure H-1. Summary of 14-Day Food Consumption and Individual Data

[1]Summary data was calculated manually from the raw data stored in the LABCAT application; numbers are in Archive, Vol. II, Raw Num Data. *$\rho \leq 0.05$

Table H-1
Protocol No. 08UG-70-IV09-06-01
Subacute Oral Toxicity of TAG-MNT in Female Rats

Summary of 14-Day Food Consumption (grams)

		Vehicle Control	TAG-MNT (mg/kg)					
Period		0	62.5	125	250	500	1000	2000
Day 0-7	Mean	139.1	135.9	135	136.4	140.7	116.6	85.2
	S.D.	9.915756	10.89801	7.874008	6.636599	11.02573	27.6132	33.44581
	N	10	10	10	10	10	10	10
Day 7-13	Mean	124.8889	118.6	120.6	126.4	122.9	122.7	111.6
	S.D.	10.25237	10.49021	9.276014	20.2166	25.77014	13.08137	15.56599
	N	9	10	10	10	10	10	5
Day 0-13	Mean	264.3333	254.5	255.6	262.8	263.6	239.3	216.6
	S.D.	20.34699	20.71097	16.34489	22.03432	31.49321	35.20432	27.91595
	N	9	10	10	10	10	10	5

Table H-2
Protocol No. 08UG-70-IV09-06-01
Subacute Oral Toxicity of TAG-MNT in Female Rats

Individual 14-Day Food Consumption (grams)

	Animal ID	Day 0-7	Day 7-13	Total
Vehicle Control	0009-10F	125	113	238
	0030-10F	136		
	0035-10F	147	135	282
	0038-10F	154	138	292
	0044-10F	136	122	258
	0061-10F	130	113	243
	0069-10F	131	112	243
	0078-10F	135	128	263
	0085-10F	144	129	273
	0087-10F	153	134	287
Mean		**139**	**125**	**264**
SD		**9.916**	**10.252**	**20.347**

	Animal ID	Day 0-7	Day 7-13	Total
62.5 mg/kg K-26	0013-10F	125	111	236
	0015-10F	143	132	275
	0016-10F	120	105	225
	0031-10F	138	109	247
	0040-10F	122	109	231
	0053-10F	136	122	258
	0066-10F	133	114	247
	0067-10F	144	127	271
	0077-10F	153	135	288
	0081-10F	145	122	267
Mean		**136**	**119**	**255**
SD		**10.898**	**10.490**	**20.711**

	Animal ID	Day 0-7	Day 7-13	Total
125 mg/kg K-26	0005-10F	133	120	253
	0029-10F	148	132	280
	0032-10F	131	114	245
	0034-10F	122	106	228
	0037-10F	126	113	239
	0043-10F	140	120	260
	0072-10F	131	122	253
	0074-10F	142	138	280
	0079-10F	137	124	261
	0090-10F	140	117	257
Mean		**135**	**121**	**256**
SD		**7.874**	**9.276**	**16.345**

Table H-2
Protocol No. 08UG-70-IV09-06-01
Subacute Oral Toxicity of TAG-MNT in Female Rats

Individual 14-Day Food Consumption (grams)

	Animal ID	Day 0-7	Day 7-13	Total
250 mg/kg K-26	0006-10F	137	121	258
	0024-10F	134	109	243
	0025-10F	150	121	271
	0036-10F	126	119	245
	0042-10F	138	182	320
	0055-10F	137	126	263
	0060-10F	130	116	246
	0070-10F	132	128	260
	0075-10F	142	120	262
	0089-10F	138	122	260
Mean		**136**	**126**	**263**
SD		**6.637**	**20.217**	**22.034**

	Animal ID	Day 0-7	Day 7-13	Total
5-F RED				
500 mg/kg K-26	0003-10F	144	60	204
	0004-10F	139	141	280
	0020-10F	135	113	248
	0021-10F	167	157	324
	0033-10F	132	124	256
	0052-10F	125	112	237
	0063-10F	139	130	269
	0065-10F	145	138	283
	0076-10F	143	127	270
	0082-10F	138	127	265
Mean		**141**	**123**	**264**
SD		**11.026**	**25.770**	**31.493**

	Animal ID	Day 0-7	Day 7-13	Total
1000 mg/kg K-26	0001-10F	113	104	217
	0007-10F	121	122	243
	0011-10F	78	121	199
	0023-10F	62	123	185
	0045-10F	132	118	250
	0048-10F	126	123	249
	0050-10F	108	103	211
	0056-10F	138	134	272
	0068-10F	144	146	290
	0084-10F	144	133	277
Mean		**117**	**123**	**239**
SD		**27.613**	**13.081**	**35.204**

Table H-2
Protocol No. 08UG-70-IV09-06-01
Subacute Oral Toxicity of TAG-MNT in Female Rats

Individual 14-Day Food Consumption (grams)

	Animal ID			
2000 mg/kg K-26	0008-10F	46	f	f
	0017-10F	50	f	f
	0046-10F	83	105	188
	0047-10F	123	126	249
	0051-10F	98	89	187
	0059-10F	27	f	f
	0071-10F	113	112	225
	0073-10F	87	f	f
	0083-10F	108	126	234
	0088-10F	117	f	f
Mean		**85.2**	**111.6**	**216.6**
SD		**33.446**	**15.566**	**27.916**

f = Animal removed from study due to severe weight loss

APPENDIX I
SUMMARY OF 14-DAY CLINICAL CHEMISTRY AND INDIVIDUAL DATA

Table I-1
Protocol No. 0BME-30-09-03-01
Subacute Oral Toxicity of TAG-MNT in Female Rats

Summary of 14-Day Clinical Chemistry

		TAG-MNT (mg/kg)						
Organ	Vehicle Control	0	62.5	125	250	500	1000	2000
ALB	Mean	3.1	3.2	3.2	3.2	3.2	3.1	2.6
(g/dL)	S.D.	0.2	0.2	0.1	0.2	0.3	0.2	0.3
ALKP	Mean	209.6	216.9	215.6	213.2	223.2	168.0	204.4
(U/L)	S.D.	42.7	47.4	35.5	26.8	41.5	52.4	60.6
ALT	Mean	50.2	48.8	43.7	39.1	43.0	31.4[a]	22.6[a]
(U/L)	S.D.	5.4	6.1	4.2	5.3	6.1	8.5	5.0
BUN	Mean	17.3	18.1	18.8	19.0	21.0	24.4[a]	25[a]
(mg/dL)	S.D.	2.9	1.7	3.4	3.3	4.6	1.8	4.8
Ca	Mean	10.4	10.1	10.4	10.4	10.4	10.3	10.1
(mg/dL)	S.D.	0.2	0.4	0.3	0.4	0.3	0.3	0.4
CHOL	Mean	44.0	49.3	42.4	48.3	48.2	37.3	33.6
(mg/dL)	S.D.	16.0	12.5	14.3	8.5	21.8	15.5	5.8
CREA	Mean	0.5	0.5	0.5	0.6	0.6	0.6[a]	0.8[a]
(mg/dL)	S.D.	0.1	0.1	0.1	0.1	0.1	0.1	0.1
GLOB	Mean	2.6	2.7	2.7	2.7	2.7	2.5	2.5
(g/dL)	S.D.	0.1	0.2	0.1	0.2	0.1	0.2	0.2
GLU	Mean	146.3	136.2	134.0	133.5	119.2[a]	119.7[a]	118[a]
(mg/dL)	S.D.	13.9	17.6	17.8	13.6	20.9	10.9	5.0
LDH	Mean	2038.9	3209.9	2423.2	1667.0	2717.1	2889.2	3565.8
(U/L)	S.D.	1038.7	1647.1	1493.2	792.5	1722.1	1508.6	2306.3
PHOS	Mean	7.7	7.5	7.6	8.1	8.4	9.4[a]	10.2[a]
(mg/dL)	S.D.	0.4	0.5	0.4	0.9	1.0	1.4	1.7
TBIL	Mean	0.1	0.3	0.2	0.1	0.2	0.3	0.3
(mg/dL)	S.D.	0.0	0.3	0.1	0.0	0.1	0.2	0.5
TP	Mean	5.7	5.9	5.9	5.9	5.9	5.6	5.1
(g/dL)	S.D.	0.2	0.3	0.2	0.3	0.3	0.3	0.4
Na	Mean	139.3	138.9	139.4	140.4	138.7	137.1	134.4
(mmol/L)	S.D.	1.3	1.8	2.3	2.4	2.2	3.0	1.1
K	Mean	4.5	4.8	4.6	4.6	4.4	4.6	5.5
(mmol/L)	S.D.	0.5	0.4	0.5	0.8	0.6	0.8	1.0
Cl	Mean	105.4	105.4	104.9	104.6	103.2	101.3	101.2
(mmol/L)	S.D.	2.2	1.0	2.0	2.0	2.5	1.6	2.7

[a]Significantly different from Vehicle-treated animals

Table I-2
Protocol No. 0BME-30-09-03-01
Subacute Oral Toxicity of TAG-MNT in Female Rats
14-Day Individual Clinical Chemistry

	Animal/Sar ID	ALB (g/dL)	ALKP (U/L)	ALT (U/L)	BUN (mg/dL)	Ca (mg/dL)	CHOL (mg/dL)	CREA (mg/dL)	GLOB (g/dL)	GLU (mg/dL)	LDH (U/L)	PHOS (mg/dL)	TBIL (mg/dL)	TP (g/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
Vehicle	10-0038	3.1	156	56	16	10.4	25	0.6	2.7	115	ND	8.1	0.2	5.8	139	5.3	108
	10-0061	3.1	269	47	13	10.7	49	0.4	2.5	142	4498	8	0.1	5.6	141	4.1	104
	10-0078	3	164	51	18	10.5	25	0.4	2.6	150	1925	7.7	0.1	5.5	141	4.2	105
	10-0009	3.1	172	61	20	10.2	56	0.4	2.6	144	2144	7.2	0.2	5.7	139	5.1	106
	10-0030	3.3	226	47	13	10.5	39	0.4	2.6	160	2110	7.3	0.1	5.8	140	3.9	106
	10-0035	3.2	191	44	20	10.7	43	0.5	2.8	152	1257	8.1	0.1	6	138	4.4	101
	10-0069	2.8	271	43	17	9.9	65	0.6	2.7	145	1998	7.5	0.1	5.5	140	4.4	109
	10-0044	3	189	50	22	10.4	42	0.5	2.6	146	1820	8.4	0.1	5.6	140	4.8	105
	10-0085	2.8	207	51	16	10.4	26	0.5	2.7	168	1931	7.3	0.1	5.5	137	4.7	105
	10-0087	3.3	251	52	18	10.5	68	0.5	2.5	141	867	7.4	0.1	5.8	138	4.2	104
	Mean	3.1	209.6	50.2	17.3	10.4	44.0	0.5	2.6	146.3	2038.9	7.7	0.1	5.7	139.3	4.5	105.4
	S.D.	0.2	42.7	5.4	2.9	0.2	16.0	0.1	0.1	13.9	1036.7	0.4	0.0	0.2	1.3	0.5	2.2

	Animal/Sar ID	ALB (g/dL)	ALKP (U/L)	ALT (U/L)	BUN (mg/dL)	Ca (mg/dL)	CHOL (mg/dL)	CREA (mg/dL)	GLOB (g/dL)	GLU (mg/dL)	LDH (U/L)	PHOS (mg/dL)	TBIL (mg/dL)	TP (g/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
62.5 mg/kg	10-0015	3.1	226	41	19	10.3	33	0.5	3	125	4186	7.7	0.3	6.1	141	4.8	106
	10-0053	3.4	167	45	19	10.2	38	0.4	2.5	113	1889	7.9	0.1	5.9	140	4	105
	10-0077	2.9	186	53	15	10	46	0.4	2.5	132	2293	7.7	0.1	5.5	140	5	106
	10-0013	3	228	54	18	9.8	30	0.5	2.6	135	2077	7.5	0.1	5.6	140	4.7	107
	10-0031	3.2	250	59	18	9.8	64	0.5	2.6	119	ND	7.5	0.2	6	135	4.6	104
	10-0066	3.1	185	48	17	10.4	46	0.4	2.6	161	1299	7	0.3	5.7	140	4.7	106
	10-0081	3.2	154	41	16	10.2	62	0.4	2.7	149	1830	6.8	0.5	5.9	138	4.9	105
	10-0016	3.1	284	54	19	9.6	59	0.5	2.9	128	5453	6.8	0.1	6	ND	ND	ND
	10-0040	3.3	292	44	21	10.3	62	0.5	2.9	167	4501	7.1	0.2	6.1	138	5.2	106
	10-0067	3.6	197	49	19	10.8	51	0.4	2.9	133	5361	8.5	0.9	6.5	138	5.4	104
	Mean	3.2	216.9	48.8	18.1	10.1	49.3	0.5	2.7	136.2	3269.9	7.5	0.3	5.9	138.9	4.8	105.4
	S.D.	0.2	47.4	6.1	1.7	0.4	12.5	0.1	0.2	17.6	1647.1	0.5	0.3	0.3	1.8	0.4	1.0

Table I-2
Protocol No. 0BME-30-09-03-01
Subacute Oral Toxicity of TAG-MNT in Female Rats
14-Day Individual Clinical Chemistry

Table I-2 (Continued)

	Animal/Sar ID	ALB (g/dL)	ALKP (U/L)	ALT (U/L)	BUN (mg/dL)	Ca (mg/dL)	CHOL (mg/dL)	CREA (mg/dL)	GLOB (g/dL)	GLU (mg/dL)	LDH (U/L)	PHOS (mg/dL)	TBIL (mg/dL)	TP (g/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
125 mg/kg	10-0005	3	211	42	16	9.9	20	0.5	2.5	118	1495	7	0.1	5.5	142	3.8	107
	10-0079	3.3	212	52	16	10.7	44	0.6	2.8	104	1526	7.7	0.1	6.1	141	4.6	105
	10-0090	3.2	205	45	19	10.6	44	0.5	2.6	139	2756	8.2	0.1	5.7	141	5	105
	10-0029	3.4	208	37	18	10.4	40	0.4	2.7	121	1006	7.6	0.2	6.1	140	4.4	103
	10-0032	3.2	195	46	16	10.5	28	0.4	2.8	151	1296	7.4	0.3	6	138	4.1	103
	10-0034	3	259	45	24	10.3	41	0.6	2.8	162	1325	7.7	0.1	5.8	138	4.9	106
	10-0043	3.4	264	39	15	10.5	40	0.6	2.9	122	5235	8.1	0.3	6.3	138	4.4	104
	10-0037	3.1	243	41	18	9.9	63	0.4	2.7	136	1736	6.9	0.2	5.8	143	4.4	109
	10-0072	3.1	219	45	22	10.4	68	0.5	2.7	136	4597	8.1	0.1	5.8	137	5.1	104
	10-0074	3.2	140	45	24	10.3	36	0.6	2.6	151	3260	7.7	0.3	5.8	136	5.2	103
	Mean	3.2	215.6	43.7	18.6	10.4	42.4	0.5	2.7	134.0	2423.2	7.6	0.2	5.9	139.4	4.6	104.9
	S.D.	0.1	35.5	4.2	3.4	0.3	14.3	0.1	0.1	17.8	1493.2	0.4	0.1	0.2	2.3	0.5	2.0

	Animal/Sar ID	ALB (g/dL)	ALKP (U/L)	ALT (U/L)	BUN (mg/dL)	Ca (mg/dL)	CHOL (mg/dL)	CREA (mg/dL)	GLOB (g/dL)	GLU (mg/dL)	LDH (U/L)	PHOS (mg/dL)	TBIL (mg/dL)	TP (g/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
250 mg/kg	10-0006	3.4	247	37	18	10.6	44	0.6	2.7	141	1870	7.7	0.1	6	142	4	106
	10-0025	3.4	203	41	17	11.4	49	0.7	3.1	106	527	10.6	0.1	6.6	145	6.6	100
	10-0075	2.9	178	37	18	10.1	59	0.5	2.7	152	1645	7.4	0.1	5.6	142	4.5	105
	10-0024	3.2	225	38	19	10.7	52	0.5	2.5	126	1177	7.6	0.2	5.7	141	4.6	106
	10-0042	3.1	215	33	18	10.2	44	0.5	2.6	142	1466	7.5	0.1	5.7	141	4.4	105
	10-0070	3.1	191	35	14	10.2	52	0.6	2.8	132	2268	7.8	0.1	5.9	138	4.3	104
	10-0089	3.2	228	42	17	10.2	63	0.5	2.6	127	926	8.4	0.1	5.7	138	4.8	104
	10-0036	2.9	183	48	24	9.9	39	0.7	2.6	144	1513	7.9	0.1	5.6	140	3.8	103
	10-0055	3	259	33	20	10.1	36	0.5	2.7	143	1862	7.8	0.1	5.8	137	4.4	107
	10-0060	3.3	203	47	25	10.3	45	0.6	2.6	122	3416	8	0.1	5.9	140	4.3	105
	Mean	3.2	213.2	39.1	19.0	10.4	48.3	0.6	2.7	133.5	1667.0	8.1	0.1	5.9	140.4	4.6	104.6
	S.D.	0.2	26.8	5.3	3.3	0.4	8.5	0.1	0.2	13.6	792.5	0.9	0.0	0.3	2.4	0.8	2.0

Table I-2
Protocol No. 0BME-30-09-03-01
Subacute Oral Toxicity of TAG-MNT in Female Rats
14-Day Individual Clinical Chemistry

Table I-2 (Continued)

	Animal/Sar ID	ALB (g/dL)	ALKP (U/L)	ALT (U/L)	BUN (mg/dL)	Ca (mg/dL)	CHOL (mg/dL)	CREA (mg/dL)	GLOB (g/dL)	GLU (mg/dL)	LDH (U/L)	PHOS (mg/dL)	TBIL (mg/dL)	TP (g/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
500 mg/kg	10-0021	3	212	40	22	10.2	20	0.6	2.8	92	5443	8.2	0.1	5.8	141	4.3	106
	10-0033	3.3	162	42	18	10.4	49	0.7	2.4	91	3011	9.4	0.1	5.7	140	4.6	105
	10-0065	3.2	210	45	27	10.4	34	0.6	2.6	122	1531	7.7	0.1	5.8	140	4	103
	10-0003	3.6	265	50	23	10.7	62	0.4	2.7	151	1446	8.2	0.2	6.3	142	4.1	103
	10-0004	3.6	274	34	19	10.6	82	0.4	2.6	111	4702	7.9	0.5	6.3	137	4.9	105
	10-0076	3.4	257	35	12	10.9	67	0.6	2.7	130	1257	8.3	0.1	6.1	136	5.5	101
	10-0020	3.1	166	45	20	9.9	21	0.5	2.6	124	1630	8.5	0.3	5.7	136	4.6	105
	10-0052	3.2	254	44	27	10.2	63	0.8	2.7	108	ND	10.3	0.2	5.9	137	3.6	98
	10-0082	2.8	207	52	21	10.3	36	0.5	2.8	144	ND	6.9	0.1	5.6	139	4.3	103
	Mean	3.2	223.2	43.0	21.0	10.4	48.2	0.6	2.7	119.2	2717.1	8.4	0.2	5.9	138.7	4.4	103.2
	S.D.	0.3	41.5	6.1	4.6	0.3	21.6	0.1	0.1	20.9	1722.1	1.0	0.1	0.3	2.2	0.6	2.5

	Animal/Sar ID	ALB (g/dL)	ALKP (U/L)	ALT (U/L)	BUN (mg/dL)	Ca (mg/dL)	CHOL (mg/dL)	CREA (mg/dL)	GLOB (g/dL)	GLU (mg/dL)	LDH (U/L)	PHOS (mg/dL)	TBIL (mg/dL)	TP (g/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
1000 mg/kg	10-0007	3.2	164	31	24	10.2	43	0.6	2.4	133	1733	7.9	0.1	5.6	141	3.9	103
	10-0011	3.3	288	22	24	10.3	32	0.6	2.4	113	5064	7.9	0.1	5.8	142	4.4	104
	10-0045	3.1	143	37	26	10.2	35	0.4	2.4	108	4492	8.9	0.1	5.6	139	4.1	101
	10-0001	2.9	110	25	22	9.9	15	0.6	2.2	106	1154	10	0.1	5.1	139	3.9	99
	10-0048	2.9	132	26	26	10.1	21	0.6	2.5	109	3177	9.9	0.4	5.4	134	4.4	101
	10-0056	3.4	216	46	23	10.8	73	0.6	2.6	137	3424	9.1	0.4	6	137	4.5	103
	10-0068	3	188	19	25	9.9	40	0.6	2.7	120	1442	7.9	0.1	5.7	136	4.4	101
	10-0023	3	125	40	28	10.7	43	0.7	2.6	129	ND	12.4	0.7	5.6	135	6	101
	10-0050	3.2	146	35	23	10.6	37	0.7	2.6	124	4184	10.5	0.4	5.8	134	6.3	100
	10-0084	2.7	168	33	23	10	34	0.6	2.3	118	1333	10	0.1	5	134	4.5	100
	Mean	3.1	168.0	31.4	24.4	10.3	37.3	0.6	2.5	119.7	2689.2	9.5	0.3	5.6	137.1	4.6	101.3
	S.D.	0.2	52.4	8.5	1.8	0.3	15.5	0.1	0.2	10.9	1508.6	1.4	0.2	0.3	3.0	0.8	1.6

Table I-2
Protocol No. 0BME-30-09-03-01
Subacute Oral Toxicity of TAG-MNT in Female Rats
14-Day Individual Clinical Chemistry

Table I-2 (Continued)

	Animal/Sar ID	ALB (g/dL)	ALKP (U/L)	ALT (U/L)	BUN (mg/dL)	Ca (mg/dL)	CHOL (mg/dL)	CREA (mg/dL)	GLOB (g/dL)	GLU (mg/dL)	LDH (U/L)	PHOS (mg/dL)	TBIL (mg/dL)	TP (g/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
2000 mg/kg	10-0046	3	137	26	27	10.4	35	0.9	2.7	123	7331	12.8	1.2	5.7	136	6.6	105
	10-0051	2.2	202	21	23	9.6	33	0.7	2.6	114	2072	9.2	0.2	4.8	134	4.5	100
	10-0083	2.6	258	16	32	10.5	42	0.9	2.3	123	1978	10.6	0.1	5	135	6.3	103
	10-0047	2.8	153	21	24	10	26	0.8	2.4	118	2196	10.1	0.1	5.2	133	5.4	99
	10-0071	2.3	274	29	19	9.8	32	0.7	2.5	112	4252	8.3	0.1	4.8	134	4.7	99
	Mean	2.6	204.4	22.6	25.0	10.1	33.6	0.8	2.5	118.0	3565.8	10.2	0.3	5.1	134.4	5.5	101.2
	S.D.	0.3	60.6	5.0	4.8	0.4	5.8	0.1	0.2	5.0	2306.3	1.7	0.5	0.4	1.1	1.0	2.7

APPENDIX J

SUMMARY OF 14-DAY CLINICAL CHEMISTRY AND INDIVIDUAL DATA

Table J-1
Protocol No. 0BME-30-09-03-01
Subacute Oral Toxicity of TAG-MNT in Female Rats

Summary of 14-Day Clinical Chemistry

		TAG-MNT (mg/kg)						
Organ	Vehicle Control	0	62.5	125	250	500	1000	2000
WBC	Mean	16.2	11.2[a]	9.3[a]	12.6	10.2[a]	7.3[a]	8.0[a]
(K/uL)	S.D.	3.2	4.4	1.8	3.2	4.3	3.2	5.7
NEU	Mean	1.3	0.8	0.8	0.7	0.8	0.5[a]	0.2[a]
(K/uL)	S.D.	1.0	0.4	0.4	0.2	0.4	0.3	0.2
(%N)	Mean	8.3	8.2	8.7	5.6	8.1	7.6	5.4
	S.D.	6.4	6.1	3.3	2.1	2.1	4.1	1.9
LYM	Mean	13.9	9.8	7.3	11.2	8.7[a]	6.2[a]	5.2[a]
(K/uL)	S.D.	3.0	4.2	2.7	3.0	3.6	2.6	5.3
(%L)	Mean	85.9	86.0	84.8	88.6	85.3	85.1	82.3
	S.D.	7.4	6.5	4.3	2.6	3.5	6.0	8.8
MONO	Mean	0.5	0.3	0.3	0.4	0.4	0.3	0.4
(K/uL)	S.D.	0.1	0.1	0.1	0.2	0.2	0.2	0.3
(%M)	Mean	2.9	2.7	3.2	3.1	3.3	4.4[a]	8.2[a]
	S.D.	0.7	0.7	0.7	0.7	1.4	1.8	3.5
EOS	Mean	0.3	0.1	0.1	0.1	0.1	0.05[a]	0.0
(K/uL)	S.D.	0.5	0.0	0.1	0.0	0.0	0.0	0.0
(%E)	Mean	1.1	1.0	1.2	0.9	1.1	0.8	0.9
	S.D.	0.3	0.3	0.4	0.4	0.4	0.4	0.6
BASO	Mean	0.3	0.2	0.2	0.2	0.2	0.2[a]	0.1
(K/uL)	S.D.	0.1	0.2	0.1	0.1	0.1	0.1	0.0
(%B)	Mean	1.9	2.1	2.1	1.8	2.2	2.1	3.2
	S.D.	0.5	0.6	0.6	0.4	0.7	0.8	2.9
RBC	Mean	7.2	7.1	7.2	7.1	7.0	7.2	7.7
(M/uL)	S.D.	0.3	0.2	0.2	0.5	0.5	0.3	0.2
HGB	Mean	14.1	13.6	14.0	13.9	13.7	13.5	14.0
(g/dL)	S.D.	0.5	0.4	0.5	1.0	0.5	0.6	0.1
HCT	Mean	41.5	40.0	41.1	41.0	40.0	40.1	41.4
(%)	S.D.	1.6	1.2	1.4	2.8	2.8	1.6	1.0
MCV	Mean	57.7	56.4	56.9	57.5	57.4	55.7	54.1
(fL)	S.D.	1.1	1.7	1.9	1.6	2.4	1.8	3.0
MCH	Mean	19.6	19.2	19.3	19.4	19.8	18.7	18.3
(pg)	S.D.	0.3	0.8	0.6	0.6	1.4	0.7	0.4
MCHC	Mean	33.9	34.0	33.9	33.8	34.4	33.6	33.9
(g/dL)	S.D.	0.3	0.5	0.2	0.2	1.6	0.5	1.1
RDW	Mean	14.4	15.1	14.2	14.9	14.2	15.3	17.4
(%)	S.D.	0.6	0.7	0.5	0.7	0.8	0.7	0.0
PLT	Mean	1073.6	1071.1	1062.6	1035.8	1084.0	848.1	1086.5
(K/uL)	S.D.	208.6	107.4	180.0	316.7	216.8	426.6	88.4
MPV	Mean	5.4	5.6	5.4	5.2	5.2	5.4	5.3
(fL)	S.D.	0.5	0.4	0.2	0.4	0.2	0.4	0.1

[a]Significantly different from Vehicle-treated animals

Table J-2
Protocol No. 0BME-30-09-03-01
Subacute Oral Toxicity of TAG-MNT in Female Rats

14-Day Individual Hematology

	Animal/Sample ID	WBC Average	WBC (K/uL)	NEU (K/uL)	NEU (%N)	LYM (K/uL)	LYM (%L)	MONO (K/uL)	MONO (%M)	EOS (K/uL)	EOS (%E)	BASO (K/uL)	BASO (%B)	RBC (M/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/uL)	MPV (fL)
Vehicle	10-0029	9.40	10.70	0.86	4.62	16.90	90.50	0.43	2.28	0.16	0.85	0.24	1.30	7.44	14.80	43.90	59.00	19.90	33.60	15.00	1221.00	5.83
	10-0061	8.95	19.50	2.09	11.80	15.00	81.95	0.58	2.99	0.21	1.09	0.44	2.24	7.26	14.50	41.90	58.40	19.40	34.30	14.70	1184.00	4.96
	10-0079	9.75	21.30	0.61	2.87	19.70	92.40	0.39	1.84	1.59	0.75	0.46	2.10	7.26	14.10	41.20	56.60	19.40	34.20	14.40	602.00	6.44
	10-0056	9.20	15.10	0.95	6.35	13.20	87.56	0.47	3.12	0.21	1.39	0.26	1.73	7.59	14.60	43.90	57.90	19.60	33.60	14.20	954.00	5.95
	10-0020	9.05	14.20	1.34	9.47	11.90	83.80	0.52	3.67	0.16	1.15	0.30	2.08	6.84	13.50	39.20	57.40	19.70	34.30	14.20	1131.00	4.97
	10-0035	8.85	18.60	1.80	9.94	15.60	84.10	0.50	3.12	0.11	0.70	0.35	2.35	7.05	13.80	40.50	57.60	19.50	33.90	14.30	1221.00	5.52
	10-0060	9.55	11.50	0.27	2.31	10.70	88.16	0.27	2.22	0.16	1.39	0.19	0.88	7.43	14.30	42.30	56.80	19.20	33.70	14.50	1108.00	5.33
	10-0044	>240	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	10-0085	8.55	13.20	0.59	4.45	11.90	90.66	0.31	2.37	0.14	1.05	0.20	1.54	7.62	13.60	40.60	57.60	19.40	33.60	13.30	1137.00	5.39
	10-0087	8.50	15.00	3.62	22.90	11.00	69.20	0.64	4.00	0.20	1.26	0.43	2.68	6.80	13.70	40.60	59.70	20.20	33.80	15.20	1624.00	5.45
	Mean	9.25	16.16	1.35	8.28	13.97	85.99	0.46	2.86	0.33	1.07	0.32	1.91	7.19	14.09	41.52	57.71	19.59	33.91	14.42	1073.56	5.40
	S.D.	0.83	3.16	1.05	6.37	3.05	7.44	0.12	0.71	0.47	0.26	0.12	0.51	0.28	0.50	1.56	1.07	0.31	0.29	0.53	208.55	0.46
	Animal/Sample ID	WBC Average	WBC (K/uL)	NEU (K/uL)	NEU (%N)	LYM (K/uL)	LYM (%L)	MONO (K/uL)	MONO (%M)	EOS (K/uL)	EOS (%E)	BASO (K/uL)	BASO (%B)	RBC (M/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/uL)	MPV (fL)
62.5 mg/kg	10-0015	13.50	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	10-0053	8.70	12.80	1.34	10.50	10.70	83.40	0.34	2.67	0.12	0.91	0.32	2.50	7.27	14.40	42.40	58.30	19.80	34.00	15.40	987.00	5.61
	10-0077	9.25	19.90	0.85	3.49	17.00	89.90	0.44	2.33	0.20	1.06	0.61	3.21	6.84	14.00	40.10	58.70	20.50	34.90	14.70	1045.00	6.00
	10-0013	10.30	7.05	0.71	10.10	5.82	82.65	0.27	3.86	0.09	1.34	0.16	2.32	6.84	13.50	39.80	59.60	19.40	34.10	15.00	1027.00	5.63
	10-0031	>900	5.18	0.35	6.85	5.44	88.16	0.15	2.98	0.09	1.34	0.13	2.09	6.67	13.20	39.60	57.90	19.40	33.50	14.20	885.00	5.86
	10-0058	9.00	12.70	0.69	5.40	11.20	88.40	0.45	3.56	0.10	0.76	0.24	1.86	7.35	13.60	40.30	54.90	18.50	34.20	15.70	1150.00	6.41
	10-0083	13.50	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	10-0016	9.80	6.30	1.40	21.20	4.62	72.30	0.19	2.96	0.09	1.43	0.09	1.46	7.39	13.20	39.80	53.90	17.80	33.10	16.40	1200.00	5.18
	10-0040	9.15	14.20	0.56	4.47	12.00	91.00	0.07	2.03	0.13	0.95	0.21	1.59	7.25	13.70	40.20	55.50	18.90	34.10	14.40	1068.00	6.22
	10-0067	ND	12.60	0.50	3.93	11.00	92.19	0.22	1.73	0.06	0.49	0.22	1.79	6.88	13.10	38.30	55.50	19.00	34.20	15.20	1157.00	5.63
	Mean	10.46	11.26	0.78	8.24	9.82	85.95	0.26	2.69	0.11	1.01	0.25	2.09	7.13	13.63	40.63	56.41	18.96	34.04	15.14	1071.13	5.81
	S.D.	1.85	4.41	0.35	6.13	4.23	5.49	0.11	0.74	0.04	0.30	0.14	0.58	0.23	0.44	1.10	1.74	0.79	0.54	0.70	107.42	0.43
	Animal/Sample ID	WBC Average	WBC (K/uL)	NEU (K/uL)	NEU (%N)	LYM (K/uL)	LYM (%L)	MONO (K/uL)	MONO (%M)	EOS (K/uL)	EOS (%E)	BASO (K/uL)	BASO (%B)	RBC (M/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/uL)	MPV (fL)
125 mg/kg	10-0005	9.60	8.55	0.73	8.15	6.75	87.56	0.22	2.65	0.05	0.59	0.19	1.14	7.18	14.10	41.60	57.10	19.60	34.40	13.20	753.00	5.62
	10-0078	9.35	10.10	0.75	7.24	9.72	85.60	0.35	3.47	0.13	1.30	0.20	2.01	6.98	14.10	42.00	60.10	20.20	33.70	14.00	1097.00	5.15
	10-0059	9.05	10.30	0.34	3.29	9.57	92.66	0.26	2.42	0.10	0.97	0.16	1.53	7.40	14.20	41.20	56.30	19.20	34.50	14.30	1247.00	5.77
	10-0028	9.10	9.40	0.29	4.42	8.78	90.69	0.19	2.65	0.05	0.78	0.09	1.44	7.17	13.90	39.90	55.60	19.30	34.80	14.00	1142.00	5.16
	10-0082	9.40	11.70	1.37	11.80	9.40	80.50	0.46	3.90	0.21	1.76	0.24	2.05	7.29	14.00	41.40	56.80	19.20	33.80	14.10	952.00	5.64
	10-0024	9.15	11.40	1.18	10.29	9.58	84.00	0.32	2.81	0.14	1.18	0.21	1.87	7.03	14.70	42.90	56.30	19.30	34.20	14.60	1096.00	5.38
	10-0043	8.65	7.33	0.66	9.31	6.09	83.16	0.29	3.95	0.08	1.02	0.26	2.67	7.42	14.60	41.20	55.50	19.60	35.30	14.40	1245.00	5.24
	10-0037	16.25	10.70	1.10	10.29	8.86	83.00	0.32	2.96	0.14	1.27	0.27	2.52	7.68	13.60	38.50	54.30	19.40	33.90	14.30	1087.00	5.44
	10-0072	8.50	8.25	1.10	13.30	6.60	79.76	0.24	2.84	0.14	1.63	0.21	2.53	7.10	13.60	39.60	56.10	19.10	34.00	15.20	1214.00	5.42
	10-0074	10.35	9.69	0.85	9.54	7.22	81.30	0.39	4.33	0.17	1.85	0.29	2.95	7.08	14.40	42.70	60.20	20.30	33.60	13.60	763.00	5.35
	Mean	9.98	9.35	0.82	8.87	7.26	84.63	0.30	3.23	0.12	1.24	0.19	2.07	7.23	13.96	41.12	56.85	19.29	34.04	14.17	1052.60	5.42
	S.D.	2.26	1.76	0.37	3.30	2.72	4.33	0.06	0.65	0.05	0.42	0.05	0.59	0.20	0.48	1.28	1.93	0.66	0.23	0.54	179.97	0.21
	Animal/Sample ID	WBC Average	WBC (K/uL)	NEU (K/uL)	NEU (%N)	LYM (K/uL)	LYM (%L)	MONO (K/uL)	MONO (%M)	EOS (K/uL)	EOS (%E)	BASO (K/uL)	BASO (%B)	RBC (M/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/uL)	MPV (fL)
250 mg/kg	10-0000	10.50	12.80	0.41	3.24	11.30	92.00	0.20	2.03	0.08	0.52	0.26	2.10	7.25	14.30	42.00	57.80	19.80	34.20	14.80	1194.00	5.24
	10-0025	9.50	18.20	0.44	2.42	16.60	91.40	0.06	3.62	0.10	0.56	0.34	2.69	7.88	15.70	46.60	59.20	19.96	33.70	16.10	1309.00	5.41
	10-0075	9.40	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	10-0024	11.45	14.10	1.10	7.82	12.00	84.80	0.54	3.86	0.20	1.42	0.29	2.15	7.69	14.20	42.10	54.68	18.40	33.70	15.00	514.00	5.72
	10-0042	9.15	12.90	0.76	5.88	11.70	90.36	0.31	2.28	0.07	0.55	0.12	0.91	7.05	14.40	42.20	59.80	20.40	34.00	15.30	1130.00	4.97
	10-0070	8.85	11.20	0.73	7.10	9.73	87.06	0.36	3.26	0.08	0.74	0.29	1.75	6.78	13.50	39.90	57.50	19.40	33.70	14.10	1253.00	4.86
	10-0069	9.50	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	10-0030	9.85	15.00	0.75	4.98	13.30	88.60	0.40	4.27	0.14	0.94	0.33	2.20	7.62	13.20	39.30	56.00	18.60	33.60	14.30	1090.00	4.80
	10-0055	9.20	9.65	0.60	6.37	8.13	85.56	0.26	2.66	0.13	1.38	0.19	2.02	6.55	12.70	37.80	57.60	19.40	33.70	15.00	899.00	5.09
	10-0060	9.10	7.80	0.38	4.65	7.02	88.90	0.28	3.57	0.09	1.07	0.12	1.56	6.99	13.20	39.90	57.40	19.40	33.90	14.20	1007.00	5.47
	Mean	9.82	12.64	0.65	5.60	11.22	88.56	0.36	3.10	0.11	0.90	0.23	1.83	7.13	13.85	41.01	57.53	19.44	33.81	14.85	1035.75	5.17
	S.D.	0.80	3.23	0.25	2.11	3.01	2.65	0.15	0.66	0.05	0.37	0.09	0.43	0.46	0.98	2.84	1.62	0.63	0.20	0.67	316.70	0.30

Table J-2
Protocol No. 0BME-30-09-03-01
Subacute Oral Toxicity of TAG-MNT in Female Rats

14-Day Individual Hematology

Table J-2 (Continued)

Animal/Sample ID	WBC (K/uL)	NEU (K/uL)	(%)	LYM (K/uL)	(%)	MONO (K/uL)	(%)	EOS (K/uL)	(%)	BASO (K/uL)	(%)	RBC (M/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/uL)	MPV (fL)
500 mg/kg																				
Average	9.25	11.00	0.63	5.06	59.66	89.30	0.36	3.05	0.19	3.67	0.19	1.54	7.19	14.26	41.95	59.66	19.90	34.00	14.10	5.31
10-0021	9.75	15.50	1.25	8.13	13.00	66.30	0.44	2.91	0.19	5.97	0.25	1.67	7.09	14.10	41.30	59.49	20.90	34.30	14.00	5.95
10-0033	9.85	14.30	1.33	9.31	11.00	66.70	0.54	5.69	0.13	1.26	0.41	2.68	7.11	14.30	42.90	59.29	20.10	33.40	14.10	5.13
10-0045		7.25	0.53	4.45	6.58	90.50	0.51	2.94	0.06	0.63	0.15	2.42	7.12	14.20	42.00	58.70	20.90	33.90	14.30	5.57
10-0057	ND	11.50	0.98	9.55	10.56	82.30	0.13	1.59	0.14	1.75	0.23	3.42	7.12	14.20	42.00	58.70	20.80	33.80	14.30	5.60
10-0069	8.70	13.30	1.31	9.55	10.56	82.30	0.58	4.59	0.13	1.63	0.42	3.56	7.17	13.40	39.70	55.30	18.10	33.80	14.00	5.59
10-0081	10.15	11.10	0.92	7.55	3.69	87.50	0.08	1.65	0.05	1.66	0.05	1.98	7.29	13.10	38.90	53.70	18.10	33.80	15.30	4.96
10-0093	8.90	4.41	0.41	3.83	3.61	91.60	0.22	1.69	0.05	1.66	0.11	2.84	7.29	13.20	39.20	54.20	18.40	33.80	14.70	4.99
10-0022	9.85	13.90	1.33	8.60	11.50	85.58	0.48	3.67	0.07	0.97	0.34	2.49	6.75	13.20	39.60	58.70	19.70	33.60	14.90	5.22
Mean	9.29	10.33	0.93	6.85	8.71	85.28	0.36	3.34	0.10	1.65	0.24	2.25	6.85	13.79	39.96	57.41	19.77	34.09	14.21	5.17
S.D.	0.63	4.30	0.41	2.15	3.92	9.48	0.25	1.45	0.05	0.41	0.13	0.69	0.45	0.48	2.57	2.55	1.44	1.70	0.76	0.21

Animal/Sample ID	WBC (K/uL)	NEU (K/uL)	(%)	LYM (K/uL)	(%)	MONO (K/uL)	(%)	EOS (K/uL)	(%)	BASO (K/uL)	(%)	RBC (M/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/uL)	MPV (fL)
1000 mg/kg																				
Average	10.00	11.09	0.66	6.20	61.96	87.30	0.32	2.91	0.07	0.66	0.35	2.19	7.15	14.00	40.60	57.00	19.60	34.20	15.20	5.42
10-0011	10.35	2.20	0.19	8.49	1.89	85.95	0.10	4.53	0.01	0.26	0.02	0.70	7.49	13.10	39.40	55.35	17.70	33.30	14.50	4.71
10-0045	10.05	10.70	0.95	8.72	9.60	84.48	0.46	4.31	0.07	0.63	0.21	1.82	7.28	13.60	41.40	56.90	18.20	33.70	15.40	5.38
10-0071	12.55	5.87	0.16	2.57	6.21	90.40	0.34	4.92	0.05	0.67	0.11	1.62	7.74	14.70	43.10	55.60	18.00	34.10	15.20	5.52
10-0046	10.10	13.30	0.95	5.45	9.20	90.20	0.20	1.62	0.10	0.95	0.15	1.47	6.68	13.20	38.90	56.20	19.70	34.10	14.10	5.49
10-0058	10.20	6.87	0.24	3.54	6.38	90.50	0.14	1.66	0.03	0.34	0.08	1.16	7.09	12.70	38.20	54.90	17.90	33.20	15.60	5.00
10-0068	9.65	10.60	0.84	7.67	3.96	82.10	0.72	6.58	0.06	0.65	0.24	3.13	6.68	13.90	39.70	57.70	19.60	34.00	15.60	5.62
10-0023	ND	4.01	0.41	10.20	3.16	78.80	0.28	6.84	0.06	1.44	0.11	2.71	7.37	13.60	41.40	56.20	18.40	32.70	14.80	23.50 ND
10-0058	11.05	4.69	0.35	4.62	6.80	90.50	0.26	4.00	0.04	0.71	0.12	2.35	7.19	13.00	39.60	53.60	18.20	33.90	16.90	5.04
10-0084	9.45	5.66	0.97	17.10	4.11	73.60	0.37	6.02	0.09	1.41	0.13	2.29	7.22	13.10	39.20	54.30	18.20	33.50	15.50	4.92
Mean	10.44	7.35	0.53	7.61	6.29	85.69	0.31	4.47	0.06	0.78	0.16	2.05	7.20	13.49	40.05	55.66	18.74	33.64	15.25	5.33
S.D.	1.02	3.20	0.31	4.10	2.83	6.03	0.18	1.84	0.03	0.37	0.11	0.82	0.29	0.59	1.55	1.61	0.75	0.48	0.72	0.38

Animal/Sample ID	WBC (K/uL)	NEU (K/uL)	(%)	LYM (K/uL)	(%)	MONO (K/uL)	(%)	EOS (K/uL)	(%)	BASO (K/uL)	(%)	RBC (M/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/uL)	MPV (fL)
2000 mg/kg																				
Average	12.46	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
10-0016	21.00	19.10	0.41	4.08	3.65	89.50	0.66	5.71	0.06	0.61	0.11	1.11	7.49	13.90	42.10	59.30	18.00	33.10	17.40	5.32
10-0051	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
10-0063	21.00	19.10	0.41	4.08	3.65	89.50	0.66	5.71	0.06	0.61	0.11	1.11	7.49	13.90	42.10	59.30	18.00	33.10	17.40	5.32
10-0047	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
10-0071	12.90	2.00	0.13	5.79	1.52	78.10	0.21	10.79	0.03	1.29	0.19	5.19	7.93	14.10	40.70	52.90	18.00	34.60	17.40	5.36
Mean	15.95	9.05	0.27	5.59	5.24	82.36	0.46	8.23	0.04	0.95	0.11	2.15	7.69	13.83	41.40	56.10	18.30	33.85	17.40	5.34
S.D.	5.16	5.73	0.29	1.66	5.25	8.77	0.26	3.62	0.03	0.47	0.01	2.68	0.34	0.34	0.99	2.97	0.42	1.06	0.00	0.09

APPENDIX K

SUMMARY OF 14-DAY ORGAN WEIGHTS AND INDIVIDUAL DATA

Table K-1
Protocol No. 0BME-30-09-03-01
Subacute Oral Toxicity of TAG-MNT in Female Rats
Summary of 14-Day Organ Weights

ABSOLUTE ORGAN WEIGHTS

Organ		Vehicle Control	TAG-MNT (mg/kg) 0	62.5	125	250	500	1000	2000
Adrenal	Mean	0.07	0.08	0.07	0.07	0.07	0.06[a]	0.05[a]
	S.D.	0.01	0.02	0.01	0.01	0.01	0.01	0.01
	N	10	10	10	10	10	10	5
Brain	Mean	1.81	1.83	1.85	1.81	1.81	1.77	1.81
	S.D.	0.10	0.08	0.07	0.06	0.05	0.10	0.09
	N	10	10	10	10	10	10	5
heart	Mean	0.80	0.83	0.82	0.81	0.81	0.69	0.76
	S.D.	0.10	0.09	0.07	0.07	0.10	0.08	0.28
	N	10	10	10	10	10	10	5
Kidney	Mean	1.65	1.67	1.67	1.70	1.78[a]	1.82[a]	1.75[a]
	S.D.	0.13	0.14	0.12	0.13	0.13	0.19	0.16
	N	10	10	10	10	10	10	5
Liver	Mean	6.66	6.60	6.68	6.77	8.03	8.75	7.89
	S.D.	0.71	0.62	0.42	0.37	0.92	0.82	0.56
	N	10	10	10	10	10	10	5
Lungs	Mean	1.22	1.13	1.10	1.17	1.20	1.11	1.14
	S.D.	0.09	0.15	0.11	0.09	0.17	0.15	0.26
	N	10	10	10	10	10	10	5
Ovaries	Mean	0.11	0.12	0.13	0.12	0.12	0.11	0.08
	S.D.	0.02	0.02	0.02	0.02	0.02	0.03	0.01
	N	10	10	10	10	10	10	5
Spleen	Mean	0.46	0.42	0.41	0.45	0.44	0.34[a]	0.31[a]
	S.D.	0.13	0.07	0.07	0.08	0.12	0.05	0.06
	N	10	10	10	10	10	10	5
thymus	Mean	0.50	0.56	0.48	0.51	0.51	0.38	0.35
	S.D.	0.19	0.16	0.10	0.08	0.12	0.10	0.15
	N	10	10	10	10	10	10	5
uterus	Mean	0.48	0.40	0.39	0.53	0.38	0.39	0.30
	S.D.	0.21	0.07	0.05	0.19	0.07	0.10	0.10
	N	10	10	10	10	10	10	5

[a]Significantly different from Vehicle

Table K-2
Protocol No. 0BME-30-09-03-01
Subacute Oral Toxicity of TAG-MNT in Female Rats
Summary of 14-Day Organ Weights

NORMALIZED ORGAN TO BODY WEIGHT RATIOS

Organ		Vehicle Control	TAG-MNT (mg/kg)					
		0	62.5	125	250	500	1000	2000
Adrenal	Mean	3.82	4.06	3.88	3.78	3.47	3.13[a]	3.33
	S.D.	0.45	0.69	0.62	0.54	0.33	0.32	0.68
	N	10	10	10	10	10	10	5
Brain	Mean	9.60	9.95	9.92	9.65	9.70	10.17	11.14[a]
	S.D.	0.71	0.40	0.44	0.62	0.69	0.57	0.71
	N	10	10	10	10	10	10	5
heart	Mean	4.26	4.49	4.40	4.31	4.33	3.97	4.64
	S.D.	0.47	0.36	0.31	0.35	0.32	0.27	1.51
	N	10	10	10	10	10	10	5
Kidney	Mean	8.73	9.05	8.90	9.06	9.55[a]	10.44[a]	10.79[a]
	S.D.	0.69	0.43	0.43	0.55	0.64	0.54	0.98
	N	10	10	10	10	10	10	5
Liver	Mean	3.52	3.59	3.57	3.60	4.28[a]	5.00[a]	4.84[a]
	S.D.	0.25	0.28	0.18	0.13	0.24	0.29	0.28
	N	10	10	10	10	10	10	5
Lungs	Mean	6.46	6.13	5.90	6.21	6.38	6.32	6.96
	S.D.	0.41	0.61	0.49	0.42	0.53	0.44	1.37
	N	10	10	10	10	10	10	5
Ovaries	Mean	5.99	6.71	7.01	6.43	6.39	6.20	5.08
	S.D.	1.20	1.17	1.03	0.92	1.07	1.58	1.08
	N	10	10	10	10	10	10	5
Spleen	Mean	2.43	2.29	2.20	2.37	2.35	1.94[a]	1.89
	S.D.	0.56	0.30	0.30	0.38	0.48	0.17	0.39
	N	10	10	10	10	10	10	5
thymus	Mean	2.65	3.04	2.56	2.73	2.74	2.17	2.14
	S.D.	0.95	0.75	0.57	0.36	0.63	0.70	0.83
	N	10	10	10	10	10	10	5
uterus	Mean	2.60	2.16	2.09	2.81	2.03	2.18	1.81
	S.D.	1.25	0.37	0.26	1.06	0.42	0.48	0.64
	N	10	10	10	10	10	10	5

[a]Significantly different from Vehicle

Table K-3
Protocol No. 0BME-30-09-03-01
Subacute Oral Toxicity of TAG-MNT in Female Rats
Summary of 14-Day Organ Weights

NORMALIZED ORGAN TO BRAIN WEIGHT RATIOS

Organ		Vehicle Control	TAG-MNT (mg/kg)					
		0	62.5	125	250	500	1000	2000
Adrenal	Mean	3.98	4.09	3.92	3.94	3.60	3.09[a]	2.97[a]
	S.D.	0.32	0.73	0.68	0.62	0.46	0.40	0.45
	N	10	10	10	10	10	10	5
Brain	Mean	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	S.D.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	N	10	10	10	10	10	10	5
heart	Mean	4.44	4.52	4.44	4.48	4.49	3.91	4.19
	S.D.	0.45	0.41	0.35	0.45	0.53	0.34	1.43
	N	10	10	10	10	10	10	5
Kidney	Mean	9.12	9.12	8.99	9.42	9.88	10.29[a]	9.70
	S.D.	0.63	0.57	0.55	0.82	0.86	0.87	0.89
	N	10	10	10	10	10	10	5
Liver	Mean	3.69	3.61	3.60	3.75	4.44[a]	4.93[a]	4.36[a]
	S.D.	0.48	0.34	0.21	0.31	0.49	0.37	0.41
	N	10	10	10	10	10	10	5
Lungs	Mean	6.74	6.18	5.95	6.46	6.63	6.23	6.26
	S.D.	0.40	0.74	0.49	0.56	0.90	0.57	1.27
	N	10	10	10	10	10	10	5
Ovaries	Mean	6.22	6.78	7.10	6.72	6.63	6.10	4.54
	S.D.	1.07	1.21	1.24	1.34	1.31	1.55	0.80
	N	10	10	10	10	10	10	5
Spleen	Mean	2.54	2.31	2.23	2.48	2.46	1.92[a]	1.69[a]
	S.D.	0.62	0.36	0.35	0.52	0.63	0.23	0.30
	N	10	10	10	10	10	10	5
thymus	Mean	2.75	3.08	2.58	2.85	2.84	1.91	1.97
	S.D.	0.95	0.82	0.56	0.45	0.68	0.91	0.88
	N	10	10	10	10	10	10	5
uterus	Mean	2.66	2.19	2.11	2.92	2.10	2.16	1.32[a]
	S.D.	1.13	0.41	0.32	1.09	0.42	0.54	0.89
	N	10	10	10	10	10	10	5

[a]Significantly different from Vehicle

Table K-4
Protocol No. 0BME-30-09-03-01
Subacute Oral Toxicity of TAG-MNT in Female Rats
14-Day Individual Organ Weights

ABSOLUTE ORGAN WEIGHTS (grams)

[a]Significantly different from Vehicle

	Animal/Sample ID	Adrenal	Brain	heart	Kidney	Liver	Lungs	Ovaries	Spleen	thymus	uterus
Vehicle	0009-10F	0.07	1.83	0.82	1.57	6.53	1.16	0.1	0.46	0.33	0.33
	0030-10F	0.07	1.78	0.8	1.62	6.4	1.2	0.13	0.46	0.42	0.43
	0035-10F	0.07	1.86	0.82	1.72	6.9	1.14	0.1	0.37	0.69	0.32
	0038-10F	0.08	1.92	1.01	1.72	6.97	1.39	0.16	0.8	0.83	0.39
	0044-10F	0.06	1.59	0.68	1.55	6.42	1.09	0.08	0.4	0.22	0.32
	0061-10F	0.08	1.79	0.9	1.63	6.3	1.18	0.12	0.42	0.52	0.94
	0069-10F	0.06	1.8	0.71	1.39	5.5	1.18	0.1	0.35	0.49	0.36
	0078-10F	0.08	1.88	0.72	1.72	6.28	1.31	0.13	0.41	0.37	0.64
	0085-10F	0.08	1.94	0.79	1.89	7.03	1.27	0.12	0.51	0.74	0.73
	0087-10F	0.07	1.7	0.79	1.67	6.23	1.27	0.09	0.43	0.42	0.38
	Mean	0.07	1.81	0.80	1.65	6.66	1.22	0.11	0.46	0.50	0.48
	S.D.	0.01	0.10	0.10	0.13	0.71	0.09	0.02	0.13	0.19	0.21

	Animal/Sample ID	Adrenal	Brain	heart	Kidney	Liver	Lungs	Ovaries	Spleen	thymus	uterus
62.5 mg/kg	0013-10F	0.08	1.81	0.76	1.44	6.01	1.02	0.14	0.4	0.28	0.33
	0015-10F	0.1	1.87	0.9	1.79	7.27	1.2	0.13	0.56	0.76	0.4
	0016-10F	0.06	1.77	0.75	1.57	5.81	0.84		0.35	0.49	
	0031-10F	0.06	1.8	0.82	1.61	7.19	1.14	0.09	0.33	0.36	0.37
	0040-10F	0.06	1.72	0.83	1.64	6.16	1.02	0.12	0.42	0.63	0.53
	0053-10F	0.09	1.92	0.92	1.84	7.24	1.22	0.13	0.36	0.5	0.37
	0066-10F	0.06	1.87	0.76	1.67	6.19	1.23	0.12	0.45	0.64	0.4
	0067-10F	0.07	1.81	0.69	1.55	6.02	1.03	0.09	0.44	0.64	0.31
	0077-10F	0.09	1.99	1.01	1.88	6.83	1.31	0.15	0.46	0.76	0.47
	0081-10F	0.08	1.73	0.83	1.69	7.32	1.3	0.15	0.46	0.58	0.43
	Mean	0.08	1.83	0.83	1.67	6.60	1.13	0.12	0.42	0.56	0.40
	S.D.	0.02	0.08	0.09	0.14	0.62	0.15	0.02	0.07	0.16	0.07

Table K-4
Protocol No. 0BME-30-09-03-01
Subacute Oral Toxicity of TAG-MNT in Female Rats
14-Day Individual Organ Weights

ABSOLUTE ORGAN WEIGHTS (grams)

Table K-4 (Continued)

	Animal/Sample ID	Adrenal	Brain	heart	Kidney	Liver	Lungs	Ovaries	Spleen	thymus	uterus
125 mg/kg	0005-10F	0.068	1.8	0.8	1.7	6.52	1.07	0.17	0.48	0.56	0.48
	0029-10F	0.1	1.87	0.94	1.69	6.86	1.17	0.13	0.38	0.47	0.45
	0032-10F	0.07	1.85	0.8	1.74	6.24	1	0.15	0.38	0.35	0.32
	0034-10F	0.06	1.83	0.71	1.52	6.47	0.96	0.1	0.4	0.43	0.33
	0037-10F	0.06	1.7	0.76	1.51	6.62	1	0.13	0.29	0.57	0.41
	0043-10F	0.06	1.85	0.84	1.6	6.23	1.08	0.11	0.38	0.33	0.38
	0072-10F	0.07	1.86	0.75	1.54	6.24	1.01		0.4	0.45	0.41
	0074-10F	0.07	1.95	0.84	1.7	6.91	1.23	0.12	0.44	0.64	0.38
	0079-10F	0.09	1.85	0.91	1.86	7.29	1.26	0.14	0.55	0.41	0.4
	0090-10F	0.08	1.97	0.87	1.79	7.38	1.25	0.13	0.43	0.57	0.34
	Mean	0.07	1.85	0.82	1.67	6.68	1.10	0.13	0.41	0.48	0.39
	S.D.	0.01	0.07	0.07	0.12	0.42	0.11	0.02	0.07	0.10	0.05

	Animal/Sample ID	Adrenal	Brain	heart	Kidney	Liver	Lungs	Ovaries	Spleen	thymus	uterus
250 mg/kg	0006-10F	0.07	1.86	0.86	1.81	6.7	1.12	0.13	0.46	0.48	0.65
	0024-10F	0.05	1.9	0.84	1.64	6.43	1.14	0.1	0.38	0.47	0.32
	0025-10F	0.07	1.84	0.86	1.85	7	1.32	0.12	0.5	0.59	0.38
	0036-10F	0.08	1.8	0.83	1.85	6.72	1.13	0.11	0.39	0.49	0.54
	0042-10F	0.07	1.69	0.87	1.79	7.01	1.17	0.16	0.47	0.52	0.76
	0055-10F	0.07	1.86	0.76	1.59	6.23	1.07	0.09	0.37	0.51	0.91
	0060-10F	0.06	1.81	0.66	1.57	6.36	1.13	0.11	0.33	0.6	0.36
	0070-10F	0.08	1.74	0.81	1.74	7.45	1.24	0.14	0.61	0.64	0.52
	0075-10F	0.08	1.79	0.72	1.49	7.05	1.06	0.13	0.52	0.41	0.42
	0089-10F	0.08	1.8	0.88	1.68	6.74	1.28	0.12	0.43	0.43	0.4
	Mean	0.07	1.81	0.81	1.70	6.77	1.17	0.12	0.45	0.51	0.53
	S.D.	0.01	0.06	0.07	0.13	0.37	0.09	0.02	0.08	0.08	0.19

Table K-4
Protocol No. 0BME-30-09-03-01
Subacute Oral Toxicity of TAG-MNT in Female Rats
14-Day Individual Organ Weights

ABSOLUTE ORGAN WEIGHTS (grams)

Table K-4 (Continued)

	Animal/Sample ID	Adrenal	Brain	heart	Kidney	Liver	Lungs	Ovaries	Spleen	thymus	uterus
500 mg/kg	0003-10F	0.08	1.79	0.76	1.95	8.21	1.43	0.12	0.4	0.57	0.54
	0004-10F	0.06	1.87	0.8	1.56	7.37	1.11	0.12	0.43	0.51	0.38
	0020-10F	0.06	1.82	0.7	1.55	6.86	1	0.11	0.32	0.61	0.3
	0021-10F	0.08	1.85	0.93	1.84	9.38	1.32	0.18	0.65	0.67	0.31
	0033-10F	0.06	1.86	0.78	1.78	7.84	1.18	0.11	0.34	0.29	0.37
	0052-10F	0.06	1.73	0.66	1.76	7.14	0.97	0.08	0.32	0.32	0.41
	0063-10F	0.06	1.79	0.94	1.84	8.92	1.22	0.13	0.4	0.47	0.35
	0065-10F	0.07	1.82	0.92	1.81	9.43	1.38	0.11	0.62	0.52	0.32
	0076-10F	0.06	1.77	0.87	1.89	7.64	1.33	0.12	0.46	0.55	0.36
	0082-10F	0.06	1.76	0.75	1.85	7.53	1.03	0.12	0.5	0.62	0.44
	Mean	0.07	1.81	0.81	1.78	8.03[a]	1.20	0.12	0.44	0.51	0.38
	S.D.	0.01	0.05	0.10	0.13	0.92	0.17	0.02	0.12	0.12	0.07

	Animal/Sample ID	Adrenal	Brain	heart	Kidney	Liver	Lungs	Ovaries	Spleen	thymus	uterus
1000 mg/kg	0001-10F	0.04	1.74	0.65	1.65	7.14	0.9	0.1	0.27	0.27	0.31
	0007-10F	0.06	1.78	0.76	1.78	9.16	1.23	0.14	0.37	0.37	0.43
	0011-10F	0.05	1.79	0.72	1.8	9.39	1.08	0.13	0.31	0.31	0.4
	0023-10F	0.05	1.61	0.54	1.45	7.84	0.93	0.09	0.27	0.56	0.14
	0045-10F	0.07	1.87	0.7	2.08	9.15	1.13	0.11	0.41	0.3	0.42
	0048-10F	0.05	1.79	0.63	1.78	8.09	1.19	0.09	0.34	0.26	0.34
	0050-10F	0.05	1.64	0.63	1.81	8.56	0.96	0.06	0.34		0.38
	0056-10F	0.06	1.85	0.78	1.88	9.28	1.24	0.12	0.37	0.4	0.46
	0068-10F	0.06	1.93	0.76	1.96	9.77	1.34	0.17	0.43	0.47	0.5
	0084-10F	0.06	1.73	0.77	2.08	9.13	1.08	0.08	0.31	0.45	0.48
	Mean	0.06[a]	1.77	0.69	1.83	8.75[a]	1.11	0.11	0.34[a]	0.38	0.39
	S.D.	0.01	0.10	0.08	0.19	0.82	0.15	0.03	0.05	0.10	0.10

Table K-4
Protocol No. 0BME-30-09-03-01
Subacute Oral Toxicity of TAG-MNT in Female Rats
14-Day Individual Organ Weights

ABSOLUTE ORGAN WEIGHTS (grams)

Table K-4 (Continued)

	Animal/Sample ID	Adrenal	Brain	heart	Kidney	Liver	Lungs	Ovaries	Spleen	thymus	uterus
2000 mg/kg	0046-10F	0.06	1.76	0.65	1.62	7.26	1.06	0.1	0.33	0.29	0.35
	0047-10F	0.04	1.7	0.61	1.84	8.48	0.93	0.07	0.2	0.51	0.29
	0051-10F	0.06	1.81	0.61	1.74	7.61	1.01	0.09	0.3	0.17	
	0071-10F	0.06	1.92	0.68	1.98	7.61	1.1	0.07	0.36		0.4
	0083-10F	0.05	1.87	1.26	1.6	8.5	1.59	0.08	0.35	0.43	0.16
	Mean	0.05[a]	1.81	0.76	1.76	7.89[a]	1.14	0.08	0.31[a]	0.35	0.30
	S.D.	0.01	0.09	0.28	0.16	0.56	0.26	0.01	0.06	0.15	0.10

[a]Significantly different from Vehicle

Table K-5
Protocol No. 0BME-30-09-03-01
Subacute Oral Toxicity of TAG-MNT in Female Rats
14-Day Individual Organ Weights

NORMALIZED ORGAN TO BODY WEIGHT RATIOS

[a]Significantly different from Vehicle

	Animal/Sample ID	Adrenal	Brain	heart	Kidney	Liver	Lungs	Ovaries	Spleen	thymus	uterus
Vehicle	0009-10F	3.78	9.89	4.43	8.49	3.53	6.27	5.41	2.49	1.78	1.78
	0030-10F	3.83	9.73	4.37	8.85	3.50	6.56	7.10	2.51	2.30	2.35
	0035-10F	3.57	9.49	4.18	8.78	3.52	5.82	5.10	1.89	3.52	1.63
	0036-10F	3.85	9.23	4.86	8.27	3.35	6.68	7.69	3.85	3.99	1.88
	0044-10F	3.24	8.59	3.68	8.38	3.47	5.89	4.32	2.16	1.19	1.73
	0061-10F	4.60	10.29	5.17	9.37	3.62	6.78	6.90	2.41	2.99	5.40
	0069-10F	3.33	10.00	3.94	7.72	3.06	6.56	5.56	1.94	2.72	2.00
	0078-10F	4.32	10.16	3.89	9.30	3.39	7.08	7.03	2.22	2.00	3.46
	0085-10F	4.26	10.32	4.20	10.05	3.74	6.76	6.38	2.71	3.94	3.88
	0087-10F	3.40	8.25	3.83	8.11	4.00	6.17	4.37	2.09	2.04	1.84
	Mean	3.817848	9.60	4.26	8.73	3.52	6.46	5.99	2.43	2.65	2.60
	S.D.	0.454601	0.71	0.47	0.69	0.25	0.41	1.20	0.56	0.95	1.25

	Animal/Sample ID	Adrenal	Brain	heart	Kidney	Liver	Lungs	Ovaries	Spleen	thymus	uterus
62.5 mg/kg	0013-10F	4.68	10.58	4.44	8.42	3.51	5.96	8.19	2.34	1.64	1.93
	0015-10F	5.15	9.64	4.64	9.23	3.75	6.19	6.70	2.89	3.92	2.06
	0016-10F	3.51	10.35	4.39	9.18	3.40	4.91		2.05	2.87	
	0031-10F	3.35	10.06	4.58	8.99	4.02	6.37	5.03	1.84	2.01	2.07
	0040-10F	3.39	9.72	4.69	9.27	3.48	5.76	6.78	2.37	3.56	2.99
	0053-10F	4.79	10.21	4.89	9.79	3.85	6.49	6.91	1.91	2.66	1.97
	0066-10F	3.28	10.22	4.15	9.13	3.38	6.72	6.56	2.46	3.50	2.19
	0067-10F	3.74	9.68	3.69	8.29	3.22	5.51	4.81	2.35	3.42	1.66
	0077-10F	4.39	9.71	4.93	9.17	3.33	6.39	7.32	2.24	3.71	2.29
	0081-10F	4.30	9.30	4.46	9.09	3.94	6.99	8.06	2.47	3.12	2.31
	Mean	4.06	9.95	4.49	9.05	3.59	6.13	6.71	2.29	3.04	2.16
	S.D.	0.69	0.40	0.36	0.43	0.28	0.61	1.17	0.30	0.75	0.37

Table K-5
Protocol No. 0BME-30-09-03-01
Subacute Oral Toxicity of TAG-MNT in Female Rats
14-Day Individual Organ Weights

NORMALIZED ORGAN TO BODY WEIGHT RATIOS

Table K-5 (Continued)

	Animal/Sample ID	Adrenal	Brain	heart	Kidney	Liver	Lungs	Ovaries	Spleen	thymus	uterus
125 mg/kg	0005-10F	3.54	9.38	4.17	8.85	3.40	5.57	8.85	2.50	2.92	2.50
	0029-10F	5.29	9.89	4.97	8.94	3.63	6.19	6.88	2.01	2.49	2.38
	0032-10F	3.83	10.11	4.37	9.51	3.41	5.46	8.20	2.08	1.91	1.75
	0034-10F	3.33	10.17	3.94	8.44	3.59	5.33	5.56	2.22	2.39	1.83
	0037-10F	3.39	9.60	4.29	8.53	3.74	5.65	7.34	1.64	3.22	2.32
	0043-10F	3.26	10.05	4.57	8.70	3.39	5.87	5.98	2.07	1.79	2.07
	0072-10F	3.74	9.95	4.01	8.24	3.34	5.40		2.14	2.41	2.19
	0074-10F	3.87	10.77	4.64	9.39	3.82	6.80	6.63	2.43	3.54	2.10
	0079-10F	4.50	9.25	4.55	9.30	3.65	6.30	7.00	2.75	2.05	2.00
	0090-10F	4.08	10.05	4.44	9.13	3.77	6.38	6.63	2.19	2.91	1.73
	Mean	3.88	9.92	4.40	8.90	3.57	5.90	7.01	2.20	2.56	2.09
	S.D.	0.62	0.44	0.31	0.43	0.18	0.49	1.03	0.30	0.57	0.26

	Animal/Sample ID	Adrenal	Brain	heart	Kidney	Liver	Lungs	Ovaries	Spleen	thymus	uterus
250 mg/kg	0006-10F	3.78	10.05	4.65	9.78	3.62	6.05	7.03	2.49	2.59	3.51
	0024-10F	2.63	10.00	4.42	8.63	3.38	6.00	5.26	2.00	2.47	1.68
	0025-10F	3.63	9.53	4.46	9.59	3.63	6.84	6.22	2.59	3.06	1.97
	0036-10F	4.23	9.52	4.39	9.79	3.56	5.98	5.62	2.06	2.59	2.86
	0042-10F	3.54	8.54	4.39	9.04	3.54	5.91	8.08	2.37	2.63	3.84
	0055-10F	3.93	10.45	4.27	8.93	3.50	6.01	5.06	2.08	2.87	5.11
	0060-10F	3.33	10.06	3.67	8.72	3.53	6.28	6.11	1.83	3.33	2.00
	0070-10F	3.96	8.66	4.03	8.66	3.71	6.17	6.97	3.03	3.18	2.59
	0075-10F	4.37	9.78	3.93	8.14	3.85	5.79	7.10	2.84	2.24	2.30
	0089-10F	4.42	9.94	4.86	9.28	3.72	7.07	6.63	2.38	2.38	2.21
	Mean	3.78	9.65	4.31	9.06	3.60	6.21	6.43	2.37	2.73	2.81
	S.D.	0.54	0.62	0.35	0.55	0.13	0.42	0.92	0.38	0.36	1.06

Table K-5
Protocol No. 0BME-30-09-03-01
Subacute Oral Toxicity of TAG-MNT in Female Rats
14-Day Individual Organ Weights

NORMALIZED ORGAN TO BODY WEIGHT RATIOS

Table K-5 (Continued)

	Animal/Sample ID	Adrenal	Brain	heart	Kidney	Liver	Lungs	Ovaries	Spleen	thymus	uterus
500 mg/kg	0003-10F	4.12	9.23	3.92	10.05	4.23	7.37	6.19	2.06	2.94	2.78
	0004-10F	3.41	10.63	4.55	8.86	4.19	6.31	6.82	2.44	2.90	2.16
	0020-10F	3.49	10.58	4.07	9.01	3.99	5.81	6.40	1.86	3.55	1.74
	0021-10F	3.92	9.07	4.56	9.02	4.60	6.47	8.82	3.19	3.28	1.52
	0033-10F	3.26	10.11	4.24	9.67	4.26	6.41	5.98	1.85	1.58	2.01
	0052-10F	3.61	10.42	3.98	10.60	4.30	5.84	4.82	1.93	1.93	2.47
	0063-10F	3.16	9.42	4.95	9.68	4.69	6.42	6.84	2.11	2.47	1.84
	0065-10F	3.33	8.67	4.38	8.62	4.49	6.57	5.24	2.95	2.48	1.52
	0076-10F	3.13	9.22	4.53	9.84	3.98	6.93	6.25	2.40	2.86	1.88
	0082-10F	3.28	9.62	4.10	10.11	4.11	5.63	6.56	2.73	3.39	2.40
	Mean	3.47	9.70	4.33	9.55	4.28	6.38	6.39	2.35	2.74	2.03
	S.D.	0.33	0.69	0.32	0.64	0.24	0.53	1.07	0.48	0.63	0.42

	Animal/Sample ID	Adrenal	Brain	heart	Kidney	Liver	Lungs	Ovaries	Spleen	thymus	uterus
1000 mg/kg	0001-10F	2.58	11.23	4.19	10.65	4.61	5.81	6.45	1.74	1.74	2.00
	0007-10F	3.35	9.94	4.25	9.94	5.12	6.87	7.62	2.07	2.07	2.40
	0011-10F	2.96	10.59	4.26	10.65	5.56	6.39	7.69	1.83	1.83	2.37
	0023-10F	3.40	10.95	3.67	9.86	5.33	6.33	6.12	1.84	3.81	0.95
	0045-10F	3.70	9.89	3.70	11.01	4.84	5.98	5.82	2.17	1.59	2.22
	0048-10F	2.86	10.23	3.60	10.17	4.62	6.80	5.14	1.94	1.49	1.94
	0050-10F	2.98	9.76	3.75	10.77	5.10	5.71	3.57	2.02		2.26
	0056-10F	3.17	9.79	4.13	9.95	4.91	6.56	6.35	1.96	2.12	2.43
	0068-10F	3.06	9.86	3.88	10.00	4.98	6.84	8.67	2.19	2.40	2.55
	0084-10F	3.30	9.51	4.23	11.43	5.02	5.93	4.40	1.70	2.47	2.64
	Mean	3.14	10.17	3.97	10.44	5.01	6.32	6.20	1.95	2.17	2.18
	S.D.	0.32	0.57	0.27	0.54	0.29	0.44	1.58	0.17	0.70	0.48

Table K-5
Protocol No. 0BME-30-09-03-01
Subacute Oral Toxicity of TAG-MNT in Female Rats
14-Day Individual Organ Weights

NORMALIZED ORGAN TO BODY WEIGHT RATIOS

Table K-5 (Continued)

	Animal/Sample ID	Adrenal	Brain	heart	Kidney	Liver	Lungs	Ovaries	Spleen	thymus	uterus
2000 mg/kg	0046-10F	3.85	11.28	4.17	10.38	4.65	6.79	6.41	2.12	1.86	2.24
	0047-10F	2.40	10.18	3.65	11.02	5.08	5.57	4.19	1.20	3.05	1.74
	0051-10F	4.03	12.15	4.09	11.68	5.11	6.78	6.04	2.01	1.14	
	0071-10F	3.51	11.23	3.98	11.58	4.45	6.43	4.09	2.11		2.34
	0083-10F	2.91	10.87	7.33	9.30	4.94	9.24	4.65	2.03	2.50	0.93
	Mean	3.34	11.14	4.64	10.79	4.85	6.96	5.08	1.89	2.14	1.81
	S.D.	0.68	0.71	1.51	0.98	0.28	1.37	1.08	0.39	0.83	0.64

[a]Significantly different from Vehicle

Table K-6
Protocol No. 0BME-30-09-03-01
Subacute Oral Toxicity of TAG-MNT in Female Rats
14-Day Individual Organ Weights

NORMALIZED ORGAN TO BRAIN WEIGHT RATIOS

[a]Significantly different from Vehicle

	Animal/Sample ID	Adrenal	Brain	heart	Kidney	Liver	Lungs	Ovaries	Spleen	thymus	uterus
Vehicle	0009-10F	3.83	1.00	4.48	8.58	3.57	6.34	5.46	2.51	1.80	1.80
	0030-10F	3.93	1.00	4.49	9.10	3.60	6.74	7.30	2.58	2.36	2.42
	0035-10F	3.76	1.00	4.41	9.25	3.71	6.13	5.38	1.99	3.71	1.72
	0038-10F	4.17	1.00	5.26	8.96	3.63	7.24	8.33	4.17	4.32	2.03
	0044-10F	3.77	1.00	4.28	9.75	4.04	6.86	5.03	2.52	1.38	2.01
	0061-10F	4.47	1.00	5.03	9.11	3.52	6.59	6.70	2.35	2.91	5.25
	0069-10F	3.33	1.00	3.94	7.72	3.06	6.56	5.56	1.94	2.72	2.00
	0078-10F	4.26	1.00	3.83	9.15	3.34	6.97	6.91	2.18	1.97	3.40
	0085-10F	4.12	1.00	4.07	9.74	3.62	6.55	6.19	2.63	3.81	3.76
	0087-10F	4.12	1.00	4.65	9.82	4.84	7.47	5.29	2.53	2.47	2.24
	Mean	3.98	1.00	4.44	9.12	3.69	6.74	6.22	2.54	2.75	2.66
	S.D.	0.32	0.00	0.45	0.63	0.48	0.40	1.07	0.62	0.95	1.13

	Animal/Sample ID	Adrenal	Brain	heart	Kidney	Liver	Lungs	Ovaries	Spleen	thymus	uterus
62.5 mg/kg	0013-10F	4.42	1.00	4.20	7.96	3.32	5.64	7.73	2.21	1.55	1.82
	0015-10F	5.35	1.00	4.81	9.57	3.89	6.42	6.95	2.99	4.06	2.14
	0016-10F	3.39	1.00	4.24	8.87	3.28	4.75		1.98	2.77	
	0031-10F	3.33	1.00	4.56	8.94	3.99	6.33	5.00	1.83	2.00	2.06
	0040-10F	3.49	1.00	4.83	9.53	3.58	5.93	6.98	2.44	3.66	3.08
	0053-10F	4.69	1.00	4.79	9.58	3.77	6.35	6.77	1.88	2.60	1.93
	0066-10F	3.21	1.00	4.06	8.93	3.31	6.58	6.42	2.41	3.42	2.14
	0067-10F	3.87	1.00	3.81	8.56	3.33	5.69	4.97	2.43	3.54	1.71
	0077-10F	4.52	1.00	5.08	9.45	3.43	6.58	7.54	2.31	3.82	2.36
	0081-10F	4.62	1.00	4.80	9.77	4.23	7.51	8.67	2.66	3.35	2.49
	Mean	4.09	1.00	4.52	9.12	3.61	6.18	6.78	2.31	3.08	2.19
	S.D.	0.73	0.00	0.41	0.57	0.34	0.74	1.21	0.36	0.82	0.41

Table K-6
Protocol No. 0BME-30-09-03-01
Subacute Oral Toxicity of TAG-MNT in Female Rats
14-Day Individual Organ Weights

NORMALIZED ORGAN TO BRAIN WEIGHT RATIOS

Table K-6 (Continued)

	Animal/Sample ID	Adrenal	Brain	heart	Kidney	Liver	Lungs	Ovaries	Spleen	thymus	uterus
125 mg/kg	0005-10F	3.78	1.00	4.44	9.44	3.62	5.94	9.44	2.67	3.11	2.67
	0029-10F	5.35	1.00	5.03	9.04	3.67	6.26	6.95	2.03	2.51	2.41
	0032-10F	3.78	1.00	4.32	9.41	3.37	5.41	8.11	2.05	1.89	1.73
	0034-10F	3.28	1.00	3.88	8.31	3.54	5.25	5.46	2.19	2.35	1.80
	0037-10F	3.53	1.00	4.47	8.88	3.89	5.88	7.65	1.71	3.35	2.41
	0043-10F	3.24	1.00	4.54	8.65	3.37	5.84	5.95	2.05	1.78	2.05
	0072-10F	3.76	1.00	4.03	8.28	3.35	5.43		2.15	2.42	2.20
	0074-10F	3.59	1.00	4.31	8.72	3.54	6.31	6.15	2.26	3.28	1.95
	0079-10F	4.86	1.00	4.92	10.05	3.94	6.81	7.57	2.97	2.22	2.16
	0090-10F	4.06	1.00	4.42	9.09	3.75	6.35	6.60	2.18	2.89	1.73
	Mean	3.92	1.00	4.44	8.99	3.60	5.95	7.10	2.23	2.58	2.11
	S.D.	0.68	0.00	0.35	0.55	0.21	0.49	1.24	0.35	0.56	0.32

	Animal/Sample ID	Adrenal	Brain	heart	Kidney	Liver	Lungs	Ovaries	Spleen	thymus	uterus
250 mg/kg	0006-10F	3.76	1.00	4.62	9.73	3.60	6.02	6.99	2.47	2.58	3.49
	0024-10F	2.63	1.00	4.42	8.63	3.38	6.00	5.26	2.00	2.47	1.68
	0025-10F	3.80	1.00	4.67	10.05	3.80	7.17	6.52	2.72	3.21	2.07
	0036-10F	4.44	1.00	4.61	10.28	3.73	6.28	6.11	2.17	2.72	3.00
	0042-10F	4.14	1.00	5.15	10.59	4.15	6.92	9.47	2.78	3.08	4.50
	0055-10F	3.76	1.00	4.09	8.55	3.35	5.75	4.84	1.99	2.74	4.89
	0060-10F	3.31	1.00	3.65	8.67	3.51	6.24	6.08	1.82	3.31	1.99
	0070-10F	4.60	1.00	4.66	10.00	4.28	7.13	8.05	3.51	3.68	2.99
	0075-10F	4.47	1.00	4.02	8.32	3.94	5.92	7.26	2.91	2.29	2.35
	0089-10F	4.44	1.00	4.89	9.33	3.74	7.11	6.67	2.39	2.39	2.22
	Mean	3.94	1.00	4.48	9.42	3.75	6.46	6.72	2.48	2.85	2.92
	S.D.	0.62	0.00	0.45	0.82	0.31	0.56	1.34	0.52	0.45	1.09

Table K-6
Protocol No. 0BME-30-09-03-01
Subacute Oral Toxicity of TAG-MNT in Female Rats
14-Day Individual Organ Weights

NORMALIZED ORGAN TO BRAIN WEIGHT RATIOS

Table K-6 (Continued)

	Animal/Sample ID	Adrenal	Brain	heart	Kidney	Liver	Lungs	Ovaries	Spleen	thymus	uterus
500 mg/kg	0003-10F	4.47	1.00	4.25	10.89	4.59	7.99	6.70	2.23	3.18	3.02
	0004-10F	3.21	1.00	4.28	8.34	3.94	5.94	6.42	2.30	2.73	2.03
	0020-10F	3.30	1.00	3.85	8.52	3.77	5.49	6.04	1.76	3.35	1.65
	0021-10F	4.32	1.00	5.03	9.95	5.07	7.14	9.73	3.51	3.62	1.68
	0033-10F	3.23	1.00	4.19	9.57	4.22	6.34	5.91	1.83	1.56	1.99
	0052-10F	3.47	1.00	3.82	10.17	4.13	5.61	4.62	1.85	1.85	2.37
	0063-10F	3.35	1.00	5.25	10.28	4.98	6.82	7.26	2.23	2.63	1.96
	0065-10F	3.85	1.00	5.05	9.95	5.18	7.58	6.04	3.41	2.86	1.76
	0076-10F	3.39	1.00	4.92	10.68	4.32	7.51	6.78	2.60	3.11	2.03
	0082-10F	3.41	1.00	4.26	10.51	4.28	5.85	6.82	2.84	3.52	2.50
	Mean	3.60	1.00	4.49	9.89	4.45	6.63	6.63	2.46	2.84	2.10
	S.D.	0.46	0.00	0.53	0.86	0.49	0.90	1.31	0.63	0.68	0.42

	Animal/Sample ID	Adrenal	Brain	heart	Kidney	Liver	Lungs	Ovaries	Spleen	thymus	uterus
1000 mg/kg	0001-10F	2.30	1.00	3.74	9.48	4.10	5.17	5.75	1.55	1.55	1.78
	0007-10F	3.37	1.00	4.27	10.00	5.15	6.91	7.87	2.08	2.08	2.42
	0011-10F	2.79	1.00	4.02	10.06	5.25	6.03	7.26	1.73	1.73	2.23
	0023-10F	3.11	1.00	3.35	9.01	4.87	5.78	5.59	1.68	3.48	0.87
	0045-10F	3.74	1.00	3.74	11.12	4.89	6.04	5.88	2.19	1.60	2.25
	0048-10F	2.79	1.00	3.52	9.94	4.52	6.65	5.03	1.90	1.45	1.90
	0050-10F	3.05	1.00	3.84	11.04	5.22	5.85	3.66	2.07	0.00	2.32
	0056-10F	3.24	1.00	4.22	10.16	5.02	6.70	6.49	2.00	2.16	2.49
	0068-10F	3.11	1.00	3.94	10.16	5.06	6.94	8.81	2.23	2.44	2.59
	0084-10F	3.47	1.00	4.45	12.02	5.28	6.24	4.62	1.79	2.60	2.77
	Mean	3.10	1.00	3.91	10.30	4.94	6.23	6.10	1.92	1.91	2.16
	S.D.	0.40	0.00	0.34	0.87	0.37	0.57	1.55	0.23	0.91	0.54

Table K-6
Protocol No. 0BME-30-09-03-01
Subacute Oral Toxicity of TAG-MNT in Female Rats
14-Day Individual Organ Weights

NORMALIZED ORGAN TO BRAIN WEIGHT RATIOS

Table K-6 (Continued)

	Animal/Sample ID	Adrenal	Brain	heart	Kidney	Liver	Lungs	Ovaries	Spleen	thymus	uterus
2000 mg/kg	0046-10F	3.41	1.00	3.69	9.20	4.13	6.02	5.68	1.88	1.65	1.99
	0047-10F	2.35	1.00	3.59	10.82	4.99	5.47	4.12	1.18	3.00	1.71
	0051-10F	3.31	1.00	3.37	9.61	4.20	5.58	4.97	1.66	0.94	0.00
	0071-10F	3.13	1.00	3.54	10.31	3.96	5.73	3.65	1.88		2.08
	0083-10F	2.67	1.00	6.74	8.56	4.55	8.50	4.28	1.87	2.30	0.86
	Mean	2.98	1.00	4.19	9.70	4.37	6.26	4.54	1.69	1.97	1.33
	S.D.	0.45	0.00	1.43	0.89	0.41	1.27	0.80	0.30	0.88	0.89

[a]Significantly different from Vehicle

APPENDIX L

HISTOPATHOLOGY REPORT

Protocol No. 0BME-30-09-03-01
Subacute Oral Toxicity of TAG-MNT in Female Rats

Pathology Report

Effects of Oral TAG 1-MeATNO2 (Triaminoguanidinium-1-methyl-5-nitriminotetrazolate—K-26) Exposure to Female Rats (*Rattus norvegicus*)

CBI Accession Number: H-10-1004
Testing Facility Protocol Number: 0BME-30-09-03-01

Histopathology Laboratory:

Comparative Biosciences, Inc.
786 Lucerne Drive
Sunnyvale, CA 94085

Testing Facility:

US Army Center for Health Promotion and Preventive Medicine
Directorate of Toxicology
5158 Blackhawk Road
Aberdeen Proving Ground, MD 21010-5403

Test Article:

TAG 1-MeATNO2
(Triaminoguanidinium-1-methyl-5-nitriminotetrazolate-K-26)

1. TABLE OF CONTENTS

2. COMPLIANCE STATEMENT

Effects of Oral TAG 1-MeATNO2 (Triaminoguanidinium-1-methyl-5-nitriminotetrazolate—K-26) Exposure to Female Rats (*Rattus norvegicus*)

CBI Accession Number: H-10-1004
Testing Facility Protocol Number: 0BME-30-09-03-01

Testing Facility:	US Army Center for Health Promotion and Preventive Medicine Directorate of Toxicology 5158 Blackhawk Road Aberdeen Proving Ground, MD 21010-5403
Histopathology Laboratory:	Comparative Biosciences, Inc. 786 Lucerne Drive Sunnyvale, CA 94085

I, the undersigned pathologist, hereby declare that this report constitutes a true and faithful account of the results of this study, to the best of my knowledge. The histopathologic phase of this study was conducted in compliance with 40 CFR (Code of Federal Regulations) part 160 and 792 "Toxic Substances Control Act" (TSCA) Good Laboratory Practice Regulations (GLP), Environmental Protection Agency guidelines and with the GLPs of the United States Food and Drug Administration (US FDA 21 CFR Part 58), with the study protocol.

Carol Meschter 3 Aug 2010

Carol Meschter, DVM, PhD, DACVP — Date
Study Pathologist

3. QUALITY ASSURANCE STATEMENT

CBI Histology Accession No.: H-10-1004
Testing Facility: US Army Center for Health Promotion and Preventative Medicine
Testing Facility Protocol No: 0BME-30-09-03-01
Study Director: Larry Williams, PhD
Protocol Title: Effects of Oral TAG 1-MeATNO2 (Triaminoguanidinium-1-methyl-5-nitriminotetrazolate—K-26) Exposure to Female Rats (*Rattus norvegicus*)

Date of Inspection	Phase Inspected	Date reported to Study Director or Management
19Jan10	Receipt of Tissue	20Jan10
19Jan10	Loading VIP with Wet Tissues	20Jan10
20Jan10	Embedding of Processed Tissues	02Mar10
20Jan10	Sectioning of Paraffin Blocks	27Jan10
21Jan10	Loading Sakura automatic Slide Stainer	02Mar10
26Feb10	Receipt of Wet Tissue	02Mar10
26Feb10	Loading VIP with Wet Tissues	02Mar10
01Mar10	Embedding of Processed Tissues	02Mar10
01Mar10	Sectioning of Paraffin Blocks	02Mar10
02Mar10	Histopathologic slide Evaluation	02Mar10
21Apr10	Histology Raw Data	22Apr10
21Apr10	Draft Pathology Report	22Apr10
03Aug10	Final Pathology Report	03Aug10

Jeanette B. Jacobs 03Aug10

Jeanette B. Jacobs, BS — Date
Quality Assurance Manager
Comparative Biosciences, Inc.

4. SUMMARY

The objective of this research was to determine the oral LD_{50}, 95% confidence intervals and slope from oral administration of K-26, and to determine if adverse effects occur from a 14-day repetitive oral exposure regime of K-26 in the female rat, i.e., derive the NOAEL and LOAEL.

In-life portion of the study was performed at the testing facility. Formalin-fixed, trimmed specimens from selected animals were submitted to Comparative Biosciences, Inc. (CBI) for microscopic evaluation by a board-certified veterinary pathologist. Tissues were processed by standard methodology, sectioned, and stained with hematoxylin and eosin (H&E). Histopathologic evaluation and report preparation was performed by Carol Meschter, DVM, PhD, DAVCP, Study Pathologist. The histopathologic phase of this study was conducted in compliance with 40 CFR (Code of Federal Regulations) part 160 and 792 "Toxic Substances Control Act" (TSCA) Good Laboratory Practices regulation (GLP), Environmental Protection Agency guidelines, with the GLPs of the United States Food and Drug Administration (US FDA 21 CFR Part 58), and with the study protocol. There were no circumstances during the histopathology phase that may have affected the quality and/or integrity of the data. This report was issued on 3 August 2010.

Histopathologic examination of kidney and liver sections from selected animals dosed with control or test article indicated that there were clear hepatotoxic, treatment-related findings due to administration of K-26 at 250, 500, 1000, and 2000 mg/kg under the conditions of this study. Toxicologically-relevant hepatic lesions included single-cell necrosis, cytoplasmic vacuolization of hepatocytes, inflammation, hepatocyte degeneration, centrilobular hypertrophy, increased mitosis, and megakaryocytic hepatocytes. These lesions were dose-related and present in some cases at 250 mg/kg and above. With respect to these lesions, the NOAEL in this study was 125 mg/kg.

In addition, two types of lesions were noted that lacked a clear dose-relationship: lymphocytic infiltration and bile duct hyperplasia. Lymphocytic infiltration was noted in the control group and at the four lowest doses, and with decreasing incidence and severity at the higher doses. Bile duct hyperplasia was present in the control group and the three lowest-dose groups, but not at the higher doses. In the absence of a compelling explanation for the apparently inverse dose-response of these lesions, they cannot be clearly attributed to treatment.

5. INTRODUCTION

The objective of this research was to determine the oral LD_{50}, 95% confidence intervals and slope from oral administration of Triaminoguanidinium-1-methyl-5-nitriminotetrazolate-K-26 (K-26), and to determine if adverse effects occur from a 14-day repetitive oral exposure regime of K-26 in the female rat, i.e., derive the NOAEL and LOAEL.

Tissues were processed through a graded series of alcohols, embedded in paraffin, microtome sectioned, and stained with hematoxylin and eosin. Tissues were evaluated by a board-certified veterinary pathologist. Histopathologic evaluation and report preparation was performed by Carol Meschter, DVM, PhD, DAVCP, Study Pathologist. The histopathologic phase of this study was conducted in compliance with 40 CFR (Code of Federal Regulations) part 160 and 792 "Toxic Substances Control Act" (TSCA) Good Laboratory Practices regulation (GLP), Environmental Protection Agency guidelines, with the GLPs of the United States Food and Drug Administration (US FDA 21 CFR Part 58), and with the study protocol. There were no circumstances during the histopathology phase that may have affected the quality and/or integrity of the data. This report was issued on 3 August 2010.

6. EXPERIMENTAL DESIGN

The study design for the 14-day repeated dose test is outlined in Table 1. According to the study documents provided, the study consisted of nine groups of 10 female rats each but selected tissues were only submitted from Groups 1–7. Groups were dosed orally (once daily for 14 days) with vehicle or with K-26 at doses of up to 2000 mg/kg. Animals were sacrificed on Day 14 and selected tissues from selected animals from Groups 1–7 were fixed in 10% formalin and submitted for histopathology.

Table 1. Summary of Experimental Design.

	In-life Phase		Histopathology Phase	
Group	No. of Animals (female)	TAG 1-MeATNO2 (mg/kg	No. of Animals	Approximate No. of Specimens/ Animal
1-F	10	0 (vehicle)	5	3
2-F	10	62.5	5	2
3-F	10	125	5	2
4-F	10	250	6	2
5-F	10	500	5	3
6-F	10	1000	5	3
7-F	10	2000	5	3

7. IN-LIFE PHASE

The in-life phase was conducted at the Testing Facility. Selected formalin-fixed tissues from selected animals submitted to CBI for processing and microscopic examination.

8. HISTOPATHOLOGY PHASE

8.1. Tissues Submitted

Liver and kidney were submitted to CBI.

8.2. Tissue Preparation

Formalin-fixed trimmed tissues were submitted, previously gross trimmed by the testing facility. Tissues were serially processed through a graded series of alcohols, oriented and embedded in paraffin, sectioned at approximately 3- to 5-µm, stained with H&E, and cover-slipped.

8.3. Tissue Evaluation

Hematoxylin eosin-stained glass slides of tissues were qualitatively examined by light microscopy by a veterinary pathologist certified by the American College of Veterinary Pathologists. The incidence and severity of the lesions were scored using the accepted industry scoring system: normal, minimal, mild, moderate, and severe. Lesions were also scored for duration (acute, subacute, and chronic) and distribution (focal, multifocal, and diffuse).

8.4. Definition of Microscopic Findings

The following nomenclature was used to describe significant histological lesions in tissues examined. A brief definition for each lesion is provided below.

<u>Liver</u>

Hepatocellular hypertrophy. An increase in hepatocyte volume or size. This change may be diffuse or demonstrate zonality. Centrilobular hypertrophy refers to hypertrophy of hepatocytes in the area surrounding the hepatic central veins, whereas periportal hypertrophy refers to hepatocytes in the vicinity of portal triads. In addition to increased hepatocyte size, there may also be some increase in hepatocyte number, including binucleate forms. In some cases, there may be a generalized increase in hepatocellular size throughout the lobule.

Vacuolar change. Vacuolar change is characterized by the presence of clear intracytoplasmic vacuoles within hepatocytes. These vacuoles may consist of numerous fine intracytoplasmic vacuoles (microvesicular, and generally regarded as more indicative of hepatocellular toxicity than macrovesicular change) or of large, single vacuoles within each cell (macrovesicular). Distribution may be singular, diffuse or zonal. In this study, vacuolar change was located primarily periportally and/or midzonally.

Inflammatory infiltrate or inflammation.

Infiltration, lymphocytic: Presence of inflammatory cells, predominantly lymphocytes, in small aggregates scattered throughout the liver, although other cell types may be present. The foci are composed primarily of lymphocytes, but small numbers of macrophages or monocytes may also be present. These foci are often located centrilobularly, but are also found to a lesser extent, in the midzonal and periportal areas, hence the "nonzonal" location.

Inflammation, acute: Presence of inflammatory cells, predominantly neutrophils.

Inflammation, granulomatous: Small granulomatous foci composed of macrophages and monocytes, in control and other groups. In rodents, the presence of small granulomatous foci in control and treated animals is often regarded as a non-specific change, although it is not always clear why this occurs (Greaves 2007).

Bile duct hyperplasia. Characterized by the presence of bile duct-like cells also referred to as oval cells. These cells are small cells with scanty, basophilic cytoplasm and pale blue oval nuclei showing a fine chromatin pattern often forming incomplete duct-like structures. There may also be increased numbers of bile ducts in and around the portal area. This change is considered to be a normal reparative response of the liver to hepatic injury when the normal proliferative capacity of existing hepatocytes is inhibited or overwhelmed.

Single-cell necrosis (apoptotic or oncocytic). Single cell (apoptotic or pre-programmed) necrosis characterized by the presence of isolated extracellular, dense eosinophilic bodies sometimes possessing pyknotic nuclear fragments. Single cell (oncocytic) necrosis characterized by the presence of necrotic cells sometimes possessing pyknotic nuclear fragments. Significant pericellular inflammation is not generally characteristic of apoptotic necrosis, but may be present, although it is common with oncocytic necrosis. Increased incidence and severity are indications of hepatic toxicity. Distribution may be singular or multifocal, and zonal. In the two high dose groups, clear necrosis of individual centrilobular hepatocytes, with variable attendant inflammation was also found.

8.5. Data

The Comparative Biosciences, Inc., Histology Accession Number H-10-1004 was assigned to this histology study. A computer file based on the study protocol was created using StarPath™ (DruQuest, International). The pathologist examined all of the submitted tissue sections by light microscopy and recorded the findings by direct entry into the StarPath™ program. Tables were generated from the data and used by the pathologist in assessment of the histopathologic findings associated with administration of the test material. Handwritten macroscopic necropsy observations were transcribed by the pathologist into StarPath™. The term on the testing facility's necropsy sheets of "No Gross Lesions Recognized" (NGLR) becomes "No Macroscopic Lesions On File" in the Starpath™ system's nomenclature.

8.6. Regulatory Status

The histopathologic phase of this study was conducted in compliance with 40 CFR (Code of Federal Regulations) part 160 and 792 "Toxic Substances Control Act" (TSCA) Good Laboratory Practices regulation (GLP), Environmental Protection Agency guidelines, with the GLPs of the United States Food and Drug Administration (US FDA 21 CFR Part 58), with the study protocol. There were no circumstances during the histopathology phase that may have affected the quality and/or integrity of the data.

9. RESULTS

9.1. Macroscopic Findings

Macroscopic findings recorded at necropsy or observed at gross trimming were entered into StarPath™. No significant macroscopic lesions related to administration of K-26 were present.

9.2. Microscopic Findings

The histopathologic lesions are presented in detail in the StarPath™ Tables.

<u>Liver</u>

Toxicologically-relevant lesions included: single-cell necrosis (apoptotic), macrocytic and microcytic cytoplasmic vacuolization of hepatocytes, acute inflammation, hepatocyte degeneration, centrilobular and periportal hepatocyte hypertrophy, megakaryocytic (enlarged hepatocytes with enlarged nuclei) hepatocytes, increased mitoses (presence of mitotic hepatocytes), lymphocytic infiltration, and bile duct hyperplasia.

Two control animals had minimal **single-cell necrosis**, but there was a clear dose-relationship with increasing incidence and severity at the three highest doses. Trace **vacuolar change** was noted in three control animals, but again there was a clear dose-relationship with increasing incidence and severity at 250 mg/kg and above. Acute **inflammation** was noted in one animal at 500 mg/kg (mild) and two animals at 2000 mg/kg (mild to moderate), but not in the control group, and is most likely test article-related. Mild to moderate **hepatocyte degeneration** was present only in one animal each of the two highest-dose groups, and this is most likely test article-related. **Centrilobular** and **periportal hypertrophy** were also dose-related, with the incidence and severity increasing with increasing dose (periportal hypertrophy was present only at the highest dose). **Megakaryocytic hepatocytes** were noted primarily at the highest dose, although minimal lesions were noted in one animal each at 250 and 500 m/kg. In addition, minimally increased **hepatocyte mitotic activity** was noted in one animal at 1000 mg/kg.

The relationship of **lymphocytic infiltration** and **bile duct hyperplasia** to dose is problematic. Lymphocytic infiltration was noted in the control group and at the four lowest doses, but with decreasing incidence at 1000 and 2000 mg/kg. Bile duct hyperplasia was present in one animal in the control group and several animals in the three lowest-dose groups (including all six animals examined at 250 mg/kg), but not at all at the higher doses. It is therefore questionable whether these lesions are attributable to the test article, even though they are often associated with the other liver lesions seen in this study.

Granulomatous inflammation present in the control and treated groups is an incidental finding not considered to be related to treatment.

Kidney:

Mild mineralization at the corticomedullary junction was present in the groups examined and considered to be an incidental finding.

10. CONCLUSION AND DISCUSSION

Initially, kidney and liver sections from selected animals in the negative control- and high-dose groups were evaluated histopathologically. Lesions associated with toxicity were found in the liver, but not the kidney. Histopathologic examination of livers from the low- and intermediate-dose groups indicated that there were clear hepatotoxic, treatment-related findings due to administration of K-26 at 250, 500, 1000, and 2000 mg/kg under the conditions of this study. Toxicologically relevant hepatic lesions included single-cell necrosis, cytoplasmic vacuolization of hepatocytes, inflammation, hepatocyte degeneration, centrilobular and periportal hypertrophy, increased mitosis, and megakaryocytic hepatocytes. These lesions were dose-related and present in some cases at 250 mg/kg and above. With respect to these lesions, the NOAEL in this study was 125 mg/kg.

In addition, two types of lesions were noted that lacked a clear dose-relationship: lymphocytic infiltration and bile duct hyperplasia. Lymphocytic infiltration was noted in the control group and at the four lowest doses, and with decreasing incidence and severity at the higher doses. Bile duct hyperplasia was present in the control group and the three lowest-dose groups (up to 250 mg/kg), but not at the higher doses. In the absence of a compelling explanation for the apparently inverse dose-response of these lesions, they cannot be clearly attributed to treatment.

11. REFERENCES

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12. ABBREVIATIONS

Relevant abbreviations used in this report and the correct scientific term are listed below.

Abbreviation	Term	Abbreviation	Term
Mg	Milligram	EPA	Environmental Protection Agency
L	Liter	CFR	Code of Federal Regulations
µ	Micro	TSCA	Toxic Substances Control Act
m	Meter	LC	Lethal Concentration
kg	Kilogram	FDA	Food and Drug Administration
LD	Lethal dose	GLP	Good Laboratory Practices
HE	Hematoxylin eosin	NBF	10% Neutral buffered formalin
NOAEL	No adverse effect level	LOAEL	Lowest observed adverse effect level
NOEL	No effect level	DACVP	Diplomate, American College of Veterinary Pathology

APPENDIX
STARPATH™ TABLES

Overall Incidence for Females

Summarized Single Tabulated Animal Report

Overall Incidence for Females
U.S. Army Center for Health Promotion and Preventive Medicine
Effects of Oral TAG 1-MeATNO2
(Triaminoguanidinium-1-methyl-5-nitriminotetrazolate-K-26) Exposure to Female Rats (Rattus norvegicus)

PROJECT NUMBER: H-10-1004/0BME-30-09-03-01 SPECIES: Sprague-Dawley Rat
Printed on 05-11-2010.

Tissue/ Diagnosis/ Modifier(s)	0 mg/kg	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg
Kidney	(5)	(0)	(0)	(0)	(5)
Infiltration, lymphocytic	0	0	0	0	2
mild	0	0	0	0	2
Mineralization	3	0	0	0	1
mild	3	0	0	0	1
Within Normal Limits	2	0	0	0	2
Liver	(5)	(5)	(5)	(6)	(5)
Congestion	0	1	0	0	1
mild	0	1	0	0	0
moderate	0	0	0	0	1
Hyperplasia, bile duct	1	3	4	6	0
trace	1	1	1	1	0
mild	0	2	2	5	0
moderate	0	0	1	0	0
Hypertrophy, centrilobular	0	0	0	0	1
mild	0	0	0	0	1
Infiltration, lymphocytic	2	5	4	6	5
trace	1	0	0	0	0
mild	1	4	3	6	5
moderate	0	1	1	0	0
Inflammation, acute	0	0	0	0	1
mild	0	0	0	0	1
Inflammation, granulomatous	4	0	0	0	1
trace	4	0	0	0	0
mild	0	0	0	0	1
Megakaryocytes present	0	0	0	1	1
trace	0	0	0	1	1
Necrosis, single cell	2	2	3	3	3
trace	2	1	3	2	1
mild	0	1	0	1	2
Vacuolar change	3	4	2	4	6
trace	3	2	2	0	0
mild	0	2	0	4	4
moderate	0	0	0	0	2

() = Number Of Animals Examined For This Tissue

Only severities are printed. (501-505)

Overall Incidence for Females (continued)
U.S. Army Center for Health Promotion and Preventive Medicine
Effects of Oral TAG 1-MeATNO2
(Triaminoguanidinium-1-methyl-5-nitriminotetrazolate-K-26) Exposure to Female Rats (Rattus norvegicus)

PROJECT NUMBER: H-10-1004/0BME-30-09-03-01 SPECIES: Sprague-Dawley Rat
Printed on 05-11-2010.

Tissue/ Diagnosis/ Modifier(s)	0 mg/kg	1000 mg/kg	2000 mg/kg
Kidney	(5)	(5)	(5)
Mineralization	3	3	3
mild	3	3	2
moderate	0	0	1
Within Normal Limits	2	2	2
Liver	(5)	(5)	(5)
Degeneration	0	1	1
mild	0	1	0
moderate	0	0	1
Hyperplasia, bile duct	1	0	0
trace	1	0	0
Hypertrophy, periportal	0	0	2
mild	0	0	1
moderate	0	0	1
Hypertrophy, centrilobular	0	3	4
mild	0	3	2
moderate	0	0	2
Infiltration, lymphocytic	2	4	2
trace	1	0	0
mild	1	4	2
Inflammation	0	0	2
mild	0	0	1
moderate	0	0	1
Inflammation, granulomatous	4	0	0
trace	4	0	0
Megakaryocytes present	0	0	3
mild	0	0	3
Mitotic activity	0	1	0
trace	0	1	0
Necrosis, single cell	2	3	5
trace	2	1	0
mild	0	0	3
moderate	0	2	2
Vacuolar change	3	5	4
trace	3	0	0
mild	0	4	1
moderate	0	1	2
severe	0	0	1

() = Number Of Animals Examined For This Tissue

Only severities are printed. (501-505)

Summarized Single Tabulated Animal Report
Individual Macroscopic and Microscopic Observations
U.S. Army Center for Health Promotion and Preventive Medicine
Effects of Oral TAG 1-MeATNO2
(Triaminoguanidinium-1-methyl-5-nitriminotetrazolate-K-26) Exposure to Female
Rats (Rattus norvegicus)

ANIMAL NUMBER: 10-0035 SEX: Female GROUP: (1) 0 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

Kidney	-Mineralization, multifocal, mild
Liver	-Inflammation, granulomatous, nonzonal, multifocal, trace Vacuolar change, microvesicular, midzonal, trace

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ANIMAL NUMBER: 10-0044 SEX: Female GROUP: (1) 0 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

Kidney	-Mineralization, multifocal, mild
Liver	-Inflammation, granulomatous, nonzonal, multifocal, trace Necrosis, single cell, nonzonal, multifocal, trace Hyperplasia, bile duct, multifocal, trace

---

ANIMAL NUMBER: 10-0069 SEX: Female GROUP: (1) 0 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

Liver	-Inflammation, granulomatous, nonzonal, multifocal, trace Vacuolar change, macrovesicular, multifocal, nonzonal, trace Necrosis, single cell, multifocal, nonzonal, apoptotic, trace

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SPECIES: Sprague-Dawley Rat
PROJECT NUMBER: H-10-1004/0BME-30-09-03-01

Summarized Single Tabulated Animal Report (continued)
Individual Macroscopic and Microscopic Observations
U.S. Army Center for Health Promotion and Preventive Medicine
Effects of Oral TAG 1-MeATNO2
(Triaminoguanidinium-1-methyl-5-nitriminotetrazolate-K-26) Exposure to Female Rats (Rattus norvegicus)

ANIMAL NUMBER: 10-0069 SEX: Female GROUP: (1) 0 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MICROSCOPIC OBSERVATIONS (continued):

The following tissues were found to be within normal limits:
Kidney.

ANIMAL NUMBER: 10-0078 SEX: Female GROUP: (1) 0 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

Kidney	-Mineralization, multifocal, mild
Liver	-Infiltration, lymphocytic, centrilobular, multifocal, mild

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ANIMAL NUMBER: 10-0085 SEX: Female GROUP: (1) 0 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

Liver	-Inflammation, granulomatous, multifocal, trace
	Vacuolar change, multifocal, macrovesicular, trace
	Infiltration, lymphocytic, centrilobular, multifocal, trace

The following tissues were found to be within normal limits:
Kidney.

SPECIES: Sprague-Dawley Rat
PROJECT NUMBER: H-10-1004/0BME-30-09-03-01

Summarized Single Tabulated Animal Report (continued)
Individual Macroscopic and Microscopic Observations
U.S. Army Center for Health Promotion and Preventive Medicine
Effects of Oral TAG 1-MeATNO2
(Triaminoguanidinium-1-methyl-5-nitriminotetrazolate-K-26) Exposure to Female Rats (Rattus norvegicus)

ANIMAL NUMBER: 10-0016 SEX: Female GROUP: (2) 62.5 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

Liver	-Infiltration, lymphocytic, multifocal, centrilobular, mild Hyperplasia, bile duct, multifocal, trace

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ANIMAL NUMBER: 10-0031 SEX: Female GROUP: (2) 62.5 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

Liver	-Hyperplasia, bile duct, multifocal, mild Infiltration, lymphocytic, nonzonal, multifocal, mild Vacuolar change, macrovesicular, nonzonal, multifocal, mild

---

ANIMAL NUMBER: 10-0053 SEX: Female GROUP: (2) 62.5 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

Liver	-Hyperplasia, bile duct, multifocal, mild Infiltration, lymphocytic, multifocal, nonzonal, mild Necrosis, single cell, oncocytic, nonzonal, multifocal, trace Vacuolar change, macrovesicular, nonzonal, multifocal, trace Vacuolar change, microvesicular, multifocal, midzonal, mild Congestion, multifocal, mild

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SPECIES: Sprague-Dawley Rat
PROJECT NUMBER: H-10-1004/0BME-30-09-03-01

Summarized Single Tabulated Animal Report (continued)
Individual Macroscopic and Microscopic Observations
U.S. Army Center for Health Promotion and Preventive Medicine
Effects of Oral TAG 1-MeATNO2
(Triaminoguanidinium-1-methyl-5-nitriminotetrazolate-K-26) Exposure to Female Rats (Rattus norvegicus)

ANIMAL NUMBER: 10-0053 SEX: Female GROUP: (2) 62.5 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MICROSCOPIC OBSERVATIONS (continued):

ANIMAL NUMBER: 10-0067 SEX: Female GROUP: (2) 62.5 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

Liver	-Infiltration, lymphocytic, multifocal, nonzonal, mild Necrosis, single cell, oncocytic, multifocal, nonzonal, mild

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ANIMAL NUMBER: 10-0081 SEX: Female GROUP: (2) 62.5 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

Liver	-Infiltration, lymphocytic, multifocal, nonzonal, moderate Vacuolar change, macrovesicular, multifocal, nonzonal, trace

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ANIMAL NUMBER: 10-0029 SEX: Female GROUP: (3) 125 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

Liver	-Hyperplasia, bile duct, multifocal, trace Infiltration, lymphocytic, nonzonal, multifocal, mild Vacuolar change, macrovesicular, midzonal, multifocal, trace

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SPECIES: Sprague-Dawley Rat
PROJECT NUMBER: H-10-1004/0BME-30-09-03-01

Summarized Single Tabulated Animal Report (continued)
Individual Macroscopic and Microscopic Observations
U.S. Army Center for Health Promotion and Preventive Medicine
Effects of Oral TAG 1-MeATNO2
(Triaminoguanidinium-1-methyl-5-nitriminotetrazolate-K-26) Exposure to Female
Rats (Rattus norvegicus)

ANIMAL NUMBER: 10-0029 SEX: Female GROUP: (3) 125 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MICROSCOPIC OBSERVATIONS (continued):

ANIMAL NUMBER: 10-0034 SEX: Female GROUP: (3) 125 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

Liver	-Infiltration, lymphocytic, multifocal, nonzonal, moderate Vacuolar change, macrovesicular, multifocal, nonzonal, trace Necrosis, single cell, apoptotic, multifocal, trace

---

ANIMAL NUMBER: 10-0043 SEX: Female GROUP: (3) 125 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

Liver	-Infiltration, lymphocytic, nonzonal, multifocal, mild Hyperplasia, bile duct, multifocal, moderate Necrosis, single cell, nonzonal, apoptotic, multifocal, trace

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ANIMAL NUMBER: 10-0074 SEX: Female GROUP: (3) 125 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

SPECIES: Sprague-Dawley Rat
PROJECT NUMBER: H-10-1004/0BME-30-09-03-01

Summarized Single Tabulated Animal Report (continued)
Individual Macroscopic and Microscopic Observations
U.S. Army Center for Health Promotion and Preventive Medicine
Effects of Oral TAG 1-MeATNO2
(Triaminoguanidinium-1-methyl-5-nitriminotetrazolate-K-26) Exposure to Female Rats (Rattus norvegicus)

ANIMAL NUMBER: 10-0074 SEX: Female GROUP: (3) 125 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MICROSCOPIC OBSERVATIONS (continued):

Liver	-Hyperplasia, bile duct, multifocal, mild Necrosis, single cell, centrilobular, multifocal, trace

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ANIMAL NUMBER: 10-0090 SEX: Female GROUP: (3) 125 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

Liver	-Hyperplasia, bile duct, multifocal, mild Infiltration, lymphocytic, nonzonal, multifocal, mild

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ANIMAL NUMBER: 10-0006 SEX: Female GROUP: (4) 250 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

Liver	-Vacuolar change, macrovesicular, nonzonal, multifocal, mild Infiltration, lymphocytic, nonzonal, multifocal, mild Megakaryocytes present, multifocal, trace Hyperplasia, bile duct, multifocal, mild Necrosis, single cell, multifocal, apoptotic, nonzonal, mild

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SPECIES: Sprague-Dawley Rat
PROJECT NUMBER: H-10-1004/0BME-30-09-03-01

Summarized Single Tabulated Animal Report (continued)
Individual Macroscopic and Microscopic Observations
U.S. Army Center for Health Promotion and Preventive Medicine
Effects of Oral TAG 1-MeATNO2
(Triaminoguanidinium-1-methyl-5-nitriminotetrazolate-K-26) Exposure to Female Rats (Rattus norvegicus)

ANIMAL NUMBER: 10-0025 SEX: Female GROUP: (4) 250 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

Liver	-Hyperplasia, bile duct, multifocal, mild Infiltration, lymphocytic, nonzonal, multifocal, mild Necrosis, single cell, oncocytic, nonzonal, trace

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ANIMAL NUMBER: 10-0042 SEX: Female GROUP: (4) 250 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

Liver	-Hyperplasia, bile duct, multifocal, mild Necrosis, single cell, nonzonal, multifocal, oncocytic, trace Infiltration, lymphocytic, nonzonal, multifocal, mild Vacuolar change, multifocal, midzonal, microvesicular, mild

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ANIMAL NUMBER: 10-0055 SEX: Female GROUP: (4) 250 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MACROSCOPIC OBSERVATIONS:

Liver	-Slightly pale.

MICROSCOPIC OBSERVATIONS:

Liver	-Hyperplasia, bile duct, multifocal, mild Infiltration, lymphocytic, multifocal, nonzonal, mild Vacuolar change, microvesicular, midzonal, multifocal, mild

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SPECIES: Sprague-Dawley Rat
PROJECT NUMBER: H-10-1004/0BME-30-09-03-01

Summarized Single Tabulated Animal Report (continued)
Individual Macroscopic and Microscopic Observations
U.S. Army Center for Health Promotion and Preventive Medicine
Effects of Oral TAG 1-MeATNO2
(Triaminoguanidinium-1-methyl-5-nitriminotetrazolate-K-26) Exposure to Female Rats (Rattus norvegicus)

ANIMAL NUMBER: 10-0060 SEX: Female GROUP: (4) 250 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:
Liver -Hyperplasia, bile duct, multifocal, mild
Infiltration, lymphocytic, multifocal, nonzonal, mild

ANIMAL NUMBER: 10-0075 SEX: Female GROUP: (4) 250 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MACROSCOPIC OBSERVATIONS:
Liver -Pale.
MICROSCOPIC OBSERVATIONS:
Liver -Hyperplasia, bile duct, multifocal, trace
Infiltration, lymphocytic, multifocal, nonzonal, mild
Vacuolar change, macrovesicular, midzonal, multifocal, mild

ANIMAL NUMBER: 10-0003 SEX: Female GROUP: (5) 500 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MACROSCOPIC OBSERVATIONS:
Kidney -Pale bilateral
Liver -Slightly mottled
MICROSCOPIC OBSERVATIONS:
Liver -Vacuolar change, macrovesicular, microvesicular, diffuse, midzonal, mild
Infiltration, lymphocytic, multifocal, periportal, mild

The following tissues were found to be within normal limits:
Kidney.

SPECIES: Sprague-Dawley Rat
PROJECT NUMBER: H-10-1004/0BME-30-09-03-01

Summarized Single Tabulated Animal Report (continued)
Individual Macroscopic and Microscopic Observations
U.S. Army Center for Health Promotion and Preventive Medicine
Effects of Oral TAG 1-MeATNO2
(Triaminoguanidinium-1-methyl-5-nitriminotetrazolate-K-26) Exposure to Female Rats (Rattus norvegicus)

ANIMAL NUMBER: 10-0021 SEX: Female GROUP: (5) 500 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

Kidney	-Infiltration, lymphocytic, multifocal, interstitial, mild
Liver	-Vacuolar change, diffuse, midzonal, microvesicular, moderate Necrosis, single cell, nonzonal, apoptotic, multifocal, mild Hypertrophy, centrilobular, multifocal, mild Infiltration, lymphocytic, nonzonal, multifocal, mild Megakaryocytes present, trace Inflammation, acute, periportal, multifocal, mild

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ANIMAL NUMBER: 10-0063 SEX: Female GROUP: (5) 500 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

Kidney	-Mineralization, multifocal, mild
Liver	-Congestion, multifocal, moderate Vacuolar change, multifocal, periportal, microvesicular, moderate Vacuolar change, macrovesicular, multifocal, periportal, mild Infiltration, lymphocytic, centrilobular, multifocal, mild Necrosis, single cell, multifocal, nonzonal, mild

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SPECIES: Sprague-Dawley Rat
PROJECT NUMBER: H-10-1004/0BME-30-09-03-01

Summarized Single Tabulated Animal Report (continued)
Individual Macroscopic and Microscopic Observations
U.S. Army Center for Health Promotion and Preventive Medicine
Effects of Oral TAG 1-MeATNO2
(Triaminoguanidinium-1-methyl-5-nitriminotetrazolate-K-26) Exposure to Female
Rats (Rattus norvegicus)

ANIMAL NUMBER: 10-0076 SEX: Female GROUP: (5) 500 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MACROSCOPIC OBSERVATIONS:

Liver	-Mottled

MICROSCOPIC OBSERVATIONS:

Kidney	-Infiltration, lymphocytic, interstitial, multifocal, mild
Liver	-Vacuolar change, multifocal, microvesicular, midzonal, mild Inflammation, granulomatous, multifocal, nonzonal, mild Infiltration, lymphocytic, nonzonal, multifocal, mild

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ANIMAL NUMBER: 10-0082 SEX: Female GROUP: (5) 500 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

Liver	-Vacuolar change, microvesicular, midzonal, multifocal, mild Infiltration, lymphocytic, multifocal, macrovesicular, mild Necrosis, single cell, apoptotic, nonzonal, multifocal, trace

The following tissues were found to be within normal limits:
Kidney.

ANIMAL NUMBER: 10-0045 SEX: Female GROUP: (6) 1000 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MACROSCOPIC OBSERVATIONS:

Liver	-Attached to diaphragm, pale

MICROSCOPIC OBSERVATIONS:

Kidney	-Mineralization, multifocal, mild
Liver	-Vacuolar change, multifocal, midzonal, microvesicular, mild Degeneration, multifocal, hepatocyte, centrilobular, mild

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SPECIES: Sprague-Dawley Rat
PROJECT NUMBER: H-10-1004/0BME-30-09-03-01

Summarized Single Tabulated Animal Report (continued)
Individual Macroscopic and Microscopic Observations
U.S. Army Center for Health Promotion and Preventive Medicine
Effects of Oral TAG 1-MeATNO2
(Triaminoguanidinium-1-methyl-5-nitriminotetrazolate-K-26) Exposure to Female Rats (Rattus norvegicus)

ANIMAL NUMBER: 10-0045 SEX: Female GROUP: (6) 1000 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MICROSCOPIC OBSERVATIONS (continued):

Liver	
(continued)	Hypertrophy, centrilobular, multifocal, mild
	Necrosis, single cell, centrilobular, multifocal, trace
	Mitotic activity, trace

ANIMAL NUMBER: 10-0050 SEX: Female GROUP: (6) 1000 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MACROSCOPIC OBSERVATIONS:	
Liver	-Pale and mottled
MICROSCOPIC OBSERVATIONS:	
Liver	-Vacuolar change, periportal, multifocal, microvesicular, mild
	Infiltration, lymphocytic, multifocal, periportal, mild
	Hypertrophy, centrilobular, multifocal, mild
	Necrosis, single cell, multifocal, nonzonal, moderate

The following tissues were found to be within normal limits:
Kidney.

ANIMAL NUMBER: 10-0056 SEX: Female GROUP: (6) 1000 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MACROSCOPIC OBSERVATIONS:	
Liver	-Pale and mottled
MICROSCOPIC OBSERVATIONS:	
Liver	-Vacuolar change, multifocal, midzonal, microvesicular, moderate
	Hypertrophy, centrilobular, multifocal, mild
	Infiltration, lymphocytic, centrilobular, multifocal, mild

The following tissues were found to be within normal limits:
Kidney.

SPECIES: Sprague-Dawley Rat
PROJECT NUMBER: H-10-1004/0BME-30-09-03-01

Summarized Single Tabulated Animal Report (continued)
Individual Macroscopic and Microscopic Observations
U.S. Army Center for Health Promotion and Preventive Medicine
Effects of Oral TAG 1-MeATNO2
(Triaminoguanidinium-1-methyl-5-nitriminotetrazolate-K-26) Exposure to Female Rats (Rattus norvegicus)

ANIMAL NUMBER: 10-0068	SEX: Female	GROUP: (6) 1000 mg/kg
Fate: (Day= 14) Scheduled Kill		Printed on 05-11-2010.
MACROSCOPIC OBSERVATIONS:		
Kidney		-Pale
Liver		-Very pale
MICROSCOPIC OBSERVATIONS:		
Kidney		-Mineralization, multifocal, mild
Liver		-Vacuolar change, periportal, multifocal, microvesicular, mild Infiltration, lymphocytic, centrilobular, multifocal, mild Necrosis, single cell, nonzonal, multifocal, moderate
ANIMAL NUMBER: 10-0084	SEX: Female	GROUP: (6) 1000 mg/kg
Fate: (Day= 14) Scheduled Kill		Printed on 05-11-2010.
MACROSCOPIC OBSERVATIONS:		
No macroscopic entries are on file.		
MICROSCOPIC OBSERVATIONS:		
Kidney		-Mineralization, multifocal, mild
Liver		-Vacuolar change, microvesicular, multifocal, periportal, mild Infiltration, lymphocytic, multifocal, centrilobular, mild
ANIMAL NUMBER: 10-0046	SEX: Female	GROUP: (7) 2000 mg/kg
Fate: (Day= 14) Scheduled Kill		Printed on 05-11-2010.
MACROSCOPIC OBSERVATIONS:		
Liver		-Pale
MICROSCOPIC OBSERVATIONS:		
Kidney		-Mineralization, multifocal, moderate
Liver		-Necrosis, single cell, nonzonal, multifocal, moderate Infiltration, lymphocytic, multifocal, nonzonal, mild Inflammation, acute, nonzonal, multifocal, mild

SPECIES: Sprague-Dawley Rat
PROJECT NUMBER: H-10-1004/0BME-30-09-03-01

Summarized Single Tabulated Animal Report (continued)
Individual Macroscopic and Microscopic Observations
U.S. Army Center for Health Promotion and Preventive Medicine
Effects of Oral TAG 1-MeATNO2
(Triaminoguanidinium-1-methyl-5-nitriminotetrazolate-K-26) Exposure to Female Rats (Rattus norvegicus)

ANIMAL NUMBER: 10-0046 SEX: Female GROUP: (7) 2000 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MICROSCOPIC OBSERVATIONS (continued):

Liver	
(continued)	Hypertrophy, centrilobular, multifocal, moderate
	Megakaryocytes present, centrilobular, multifocal, mild

ANIMAL NUMBER: 10-0047 SEX: Female GROUP: (7) 2000 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MACROSCOPIC OBSERVATIONS:	
Liver	-Pale and mottled
MICROSCOPIC OBSERVATIONS:	
Kidney	-Mineralization, multifocal, mild
Liver	-Vacuolar change, multifocal, periportal, microvesicular, mild
	Necrosis, single cell, centrilobular, multifocal, mild
	Hypertrophy, centrilobular, multifocal, mild
	Megakaryocytes present, multifocal, mild
	Infiltration, lymphocytic, centrilobular, multifocal, mild

ANIMAL NUMBER: 10-0051 SEX: Female GROUP: (7) 2000 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MACROSCOPIC OBSERVATIONS:	
Liver	-Pale and mottled
MICROSCOPIC OBSERVATIONS:	
Liver	-Vacuolar change, multifocal, nonzonal, microvesicular, moderate
	Necrosis, single cell, nonzonal, multifocal, mild
	Hypertrophy, centrilobular, multifocal, mild

The following tissues were found to be within normal limits:
Kidney.

SPECIES: Sprague-Dawley Rat
PROJECT NUMBER: H-10-1004/0BME-30-09-03-01

Summarized Single Tabulated Animal Report (continued)
Individual Macroscopic and Microscopic Observations
U.S. Army Center for Health Promotion and Preventive Medicine
Effects of Oral TAG 1-MeATNO2
(Triaminoguanidinium-1-methyl-5-nitriminotetrazolate-K-26) Exposure to Female Rats (Rattus norvegicus)

ANIMAL NUMBER: 10-0071 SEX: Female GROUP: (7) 2000 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MACROSCOPIC OBSERVATIONS:	
Liver	-Pale and mottled
MICROSCOPIC OBSERVATIONS:	
Liver	-Necrosis, single cell, multifocal, nonzonal, moderate Vacuolar change, microvesicular, diffuse, nonzonal, severe Hypertrophy, multifocal, hepatocyte, periportal, moderate Hypertrophy, centrilobular, multifocal, moderate Inflammation, acute, multifocal, centrilobular, moderate

The following tissues were found to be within normal limits:
Kidney.

ANIMAL NUMBER: 10-0083 SEX: Female GROUP: (7) 2000 mg/kg
Fate: (Day= 14) Scheduled Kill Printed on 05-11-2010.

MACROSCOPIC OBSERVATIONS:	
Liver	-Pale
MICROSCOPIC OBSERVATIONS:	
Kidney	-Mineralization, multifocal, mild
Liver	-Vacuolar change, multifocal, microvesicular, nonzonal, moderate Hypertrophy, hepatocyte, multifocal, nonzonal, mild Necrosis, single cell, nonzonal, multifocal, mild Degeneration, midzonal, hepatocyte, multifocal, moderate Megakaryocytes present, multifocal, mild

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SPECIES: Sprague-Dawley Rat
PROJECT NUMBER: H-10-1004/0BME-30-09-03-01

APPENDIX M

STUDY PROTOCOL AND MODIFICATIONS

Protocol No. 0BME-30-09-03-01
Subacute Oral Toxicity of TAG-MNT in Female Rats

ANIMAL USE PROTOCOL
TOXICOLOGY DIRECTORATE
U.S. ARMY CENTER FOR HEALTH PROMOTION
AND PREVENTIVE MEDICINE
ABERDEEN PROVING GROUND, MD 21010-5403

PROTOCOL TITLE: Effects of Oral TAG 1-MeATNO2 (Triaminoguanidinium-1-methyl-5-nitriminotetrazolate – K-26) Exposure to Female Rats (*Rattus norvegicus*)

PROTOCOL NUMBER: ØBME -3Ø-Ø9-Ø3-Ø1

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Animal Use Protocol – Effects of Oral TAG 1-MeATNO2 (Triaminoguanidinium-1-methyl-5-nitriminotetrazolate – K-26) Exposure to Female Rats (*Rattus norvegicus*)

SPONSOR: U.S. Army Research Development Engineering Command
(AMSRD-MSF)
Environmental Acquisition and Logistics Sustainment Program
Aberdeen Proving Ground, MD 21010

I. NON-TECHNICAL SYNOPSIS:

The purpose of this study is to assess the toxicity of a new experimental explosive, triaminoguanidinium-1-methyl-5-nitriminotetrazolate (TAG 1-MeATNO2 – K-26) in the female rat after single and short-term repetitive oral exposures. Rats are widely used as experimental subjects for toxicity studies and females will be used in this study as they are deemed more sensitive to environmental toxins than males. K-26 is being considered as a replacement explosive for trinitrotriazine (RDX). In this study, laboratory rats will be used to determine an LD_{50} value, as well as estimated values for a No Observed Adverse Effect Level (NOAEL) and a Lowest Observed Adverse Effect Level (LOAEL) for K-26. The potential carcinogenicity of K-26 will be determined from blood samples taken from the animals. This study will determine how the mammalian toxicity of this experimental explosive compares to RDX and other explosives being considered to replace RDX.

II. BACKGROUND:

II.1. Background: RDX, the Army's long-standing primary explosive, is accumulating in the environment surrounding training ranges and is continuously being reassessed for health effects arising from exposure in soil, surface water, and ground water. RDX is a known environmental toxicant with an Environmental Protection Agency (EPA) acute oral minimum risk level (MRL) of 0.06 mg/kg/day based on its epileptiform seizure neurotoxicity in humans and rodents, and a Reference Dose (RfD) of 3 μg/kg/day based on prostatic inflammation in rodents. RDX is also classified as a possible carcinogen.

The Army Environmental Quality Technology (EQT) Ordnance Environmental Program (OEP) is dedicated to finding replacements for RDX that will reduce or eliminate the health risks from environmental exposure and will reduce adverse environmental safety and occupational health (ESOH) effects; RDX adversely affects the readiness and costs associated with training [(1)]. By identifying unacceptable ESOH effects early in the acquisition process, unacceptable replacements can be identified and unnecessary budget expenditures can be greatly reduced.

In collaboration with U.S. Army Research, Development, and Engineering Command (RDECOM), personnel in the Department of Chemistry and Biochemistry, Ludwig-Maximilian University, Munich, Germany, have synthesized several energetic compounds as possible replacements for RDX; K-26 is one of these compounds.

CHPPM has started evaluation of the toxicity for K-26. K-26 was found to not be toxic after 30 min exposure in the MicroTox® luminescent bacteria assay, but was toxic in the 48 hr neutral red uptake human liver cell line assay with an IC_{50} of 316 μg/ml [(16)]. Using the formula derived from the National Institute of Health consortium [(17)] the IC_{50} provides an approximate *in vivo* LD_{50} of 900 mg/kg in rat [(16)].

II.2. Literature Search for Duplication:

II.2.1. Literature Source(s) Searched:

MEDLINE, TOXFILE, FEDRIP, BIOSIS, EMBASE, CA SEARCH, DTIC, BRD

II.2.2. Date of Search: February 13, 2009

II.2.3. Period of Search: 1950 - 2009

II.2.4. Key Words of Search: triaminoguanidinium-methyl-5-nitriminotetrazolate, TAG 1-MeATNO2, explosive, toxic, LD50, rats

II.2.5. Results of Search: Three references were found searching for TAG 1-MeATNO2 describing the synthesis of K-26. The search revealed no toxicity studies on K-26. Expanding the search to include the word, "explosive", 20 articles were found, all of which dealt with toxicity studies on compounds other than K-26. Thus, our study will not be a duplication of effort.

III. OBJECTIVE/HYPOTHESIS:

The objective of this research is to determine the oral LD_{50}, 95% confidence intervals and slope constant from oral administration of K-26, and to determine if adverse effects occur from a 14-day repetitive oral exposure regime of K-26 in the female rat, i.e., derive the NOAEL and LOAEL.

IV. MILITARY RELEVANCE:

A functional, effective, quality engineered warhead formulation comprised of environmentally viable alternative substances can make a positive contribution to current and future Army readiness by being less toxic to the environment and human health. Through reduced environmental compliance constraints, a safer, more environmentally benign formulation can increase life-cycle cost effectiveness. Current formulations that use RDX have contributed to environmental contamination at sites and have closed or curtailed range operations at some locations, primarily from low order or incomplete detonations. It is imperative that the Department of the Army train soldiers in the same manner at which they fight, which requires the use of weapons using alternative replacement energetics. The acute and sub-acute toxicity tests proposed in this protocol can be used as a useful screening tool to provide support in developing less toxic and prevalent munition alternatives [1].

V. MATERIALS AND METHODS:

V.1. Experimental Design and General Procedures: The acute toxicity of K-26 will be determined in female rats. In Experiment 1, K-26 will be administered by oral gavage in a single dose of increasing concentrations to determine the LD50 using the sequential stage-wise probit

(SSWP) method. In Experiment 2, rats will be dosed daily by oral gavage at six different doses (derived from the SSWP) for 14 days. Untreated and vehicle-treated animals will be included as controls. The study end-point is euthanasia either when the rats become moribund due to compound toxicity or 14 days after treatment initiation, i.e., at the end of the study. Blood and tissue samples will be collected at the end of the study for analysis of mutagenicity of red blood cells (micronucleus assay), blood chemistry values, and histopathology.

V.1.1. Experiment 1: LD_{50} Determination:

The objective(s) of this study is to determine the acute oral LD_{50} and slope constant of K-26 in the female Sprague-Dawley rat and to set dosage levels for the subacute (14-day) study. The general procedures of this acute study will follow the EPA Health Effects Test Guidelines for Acute Oral Toxicity (OPPTS 870.1100) [2]. All oral dosing will be administered using an oral gavage needle (16 – 18 ga. x 2 in); maximum volume will not exceed 10 ml/kg [2].

A common method for LD_{50} determination is the Approximate Lethal Dose Procedure that involves dosing one female rat at a time with a minimum of 48 hours until dosing the next animal in progression. For chemicals with unknown toxicity, the procedure guidelines suggest starting at 175 mg/kg with a dose progression factor of 3.2x (1/2 log interval). However, there is no historical *in vivo* data on K-26, and thus we will use the SSWP variation for the LD_{50} determination. This procedure will involve dosing up to seven female rats with each animal receiving a different oral dose. This will facilitate the use of more dose groups in an attempt to bracket the oral toxicity of K-26 in the first stage of acute dosing. Based on reported toxicity of K-26 in the neutral red cytotoxicity assay, the LD_{50} of K-26 is estimated as being 900 mg/kg [16]; this will be the starting dose. Seven dose intervals will used set at ± 1.5x the previous dose up to a maximum of approximately 2000 mg/kg (i.e., 180, 270, 400, 600, 900, 1350, and 2000 mg/kg). K-26 has water solubility of only 98.3 mg/ml; suspensions of K-26 will be made in a suitable vehicle, e.g., saline or methylcellulose. It may still be necessary to give the higher dose animals multiple doses within a 24-hour period to stay within the 10 ml/kg maximum oral dosage volume [2].

Based on the response in the first stage, doses will be selected for the second stage surrounding the dose where lethality is observed. In the second stage, 3-5 doses will be selected based on the responses observed in the first stage and 2-3 animals will be used at each dose. If stage three is needed, a similar approach will be used. Dosing will stop when either the ratio of the confidence limit interval divided by 2 times the estimated LD_{50} is less than 0.40 or 30 animals have been used, whichever comes first. The ratio is determined after each stage of dosing. These will be discussed with the statistician for confirmation. If no deaths are observed at the highest dose level (2000 mg/kg) in the first round of dosing, the remainder of the study will be based on the limit test provision. Three consecutive animals must survive at the upper dose limit for the LD_{50} value to be reported as greater than the upper dose limit of 2000 mg/kg. This process will continue until an accurate LD_{50} is obtained; it is expected that no more than **30 animals total** will be required.

The SSWP method allows flexibility regarding dosing and the number of animals at each

stage, and provides a more accurate estimate of the LD_{50} than the Approximate Lethal Dose Procedure typically performed by DTOX. Although mortality from the 14-day observation period will be the criterion used for the analysis, time to death or time determined that individuals are unlikely to recover may also be used to make decisions regarding doses for subsequent stages. The results of the SSWP method will be used to determine the exposures for a longer 14-day range finding regime.

The LD_{50} determination will use up to 30 female Sprague Dawley rats (**n=30,** see Table 1). If fewer than 30 rats provide sufficient results, the remaining animals will be transferred for training purposes or humanely euthanized per TOX SOP 066 [3]. An appropriate vehicle, e.g., saline or methylcellulose, will be determined based on the solubility characteristics of K-26 and the concentration needed based on fixed volume amounts required to minimize diluent effects. All surviving animals will be euthanized and will undergo necropsy at the end of the 14-day observation period.

V.1.2. Experiment 2: 14-Day Repeated Dose Test

The purpose of the 14-day study is to determine if there are adverse effects from short-term repetitive oral exposures. If the results from this test suggest significant effects occur, then a subchronic testing regime may be recommended. This test will follow DTOX SOP No. 037 [4] except for the addition of positive and untreated control groups, genotoxicity assays conducted with tissue collected at necropsy, and additional organ histology, if warranted. Briefly, six dose groups, consisting of ten female rats per dose group, as well as a vehicle control group, a positive control group, and an untreated control group (**n=9 groups X 10 rats each=90,** see Table 1), will be orally dosed for 14 days. The test compound will be dissolved/suspended in an appropriate vehicle, e.g., saline or methylcellulose, and administered via oral gavage using a 16-gauge feeding needle. Dose selection will depend on the results of the SSWP done in Experiment 1 (e.g., 1x, 0.75x, 0.5x, 0.25x, 0.125x, 0.0625x, 0.03125x the LD_{50}). The vehicle control group will receive a volume of vehicle equivalent to the volume used for the highest exposure group. For all oral dosing, the maximum volume will not exceed 10 ml/kg [2]. The micronucleus positive control group will receive a known genotoxic agent (ethyl methanesulfonate @ 200 mg/kg; CAS 62-50-0) in an appropriate vehicle (e.g. saline, 0.5% w/v sodium carboxymethylcellulose solution, water, or corn oil). Positive control animals will be orally gavaged once per day for the last two days and then will be bled, euthanized, and necropsied 2-6 hours after the second dose. Untreated animals are also included in this study as controls for the micronucleus assay, but will not be dosed during the exposure period.

On day 14 of the exposure, blood will be collected from the lateral saphenous vein for the micronucleus assay (see below). Animals will be anesthetized with isoflurane, bled, euthanized with CO_2, and necropsied. The following tissues will be harvested and weighed: brain, heart, kidneys, adrenals, lungs, liver, spleen, and thymus. All gross pathology changes will be recorded on the necropsy report form (CHPPM Form 333).

Clinical chemistry and hematology will be conducted, if blood sample volumes are adequate, according to DTOX SOP No. 034 Clinical Chemistry Analysis of Blood Specimens and DTOX

SOP No. 001 Cell-Dyn 3700 Hematology Analyzer. Terminal blood will be collected per DTOX SOP No. 053. A 1-3 ml aliquot of blood will be collected via cardiac puncture (a terminal blood draw) following anesthesia with isoflurane. A portion of blood will be transferred to a 1.3 ml EDTA microtube and evaluated for total red blood cell and white blood cell counts, packed cell volume, hemoglobin, and five-part differential. Remaining blood will be transferred to a 1.3 ml serum-gel microtube and evaluated for the following chemistries: prothrombin, urea (BUN), creatinine (CREA), glucose (GLU), total protein (TP), albumin (ALB), cholesterol (CHOL), lactate dehydrogenase (LDH), total bilirubin (TBIL), calcium (CA), inorganic phosphate (PHOS), electrolytes and/or other chemical parameters that may be affected by the test compound being investigated.

Table 1

TEST	ANIMALS
ACUTE STUDY	Females
LD_{50}	30
14-DAY STUDY:	Females
Control (0mg/kg)	10
Dose TBD	10
Dose TBD	10
Dose TBD	10
Dose TBD	10
Dose TBD	10
Dose TBD	10
MN Positive Control	10
Untreated Control	10
Total	**120**

Micronucleus Assay

The micronucleus assay will be conducted using the MicroFlow Plus Kit® (Litron Laboratories) following the instructions provided with the kit [5]. Briefly, approximately 60-120 µl (approximately 3 drops) of peripheral blood will be collected from the lateral saphenous vein. Rats will be immobilized, the hindleg extended and immobilized, the skin surface coated with a lubricant (ophthalmic ointment) to aid in visualization of the vein and to reduce clotting, and the vein punctured with a sterile 16-18 gauge needle. Blood will be collected either using a pipette, which has been pre-wetted with anti-coagulant, to capture drops that form at the puncture site or allowing blood to drip into a tube containing anti-coagulant. The most important considerations are that blood is free flowing and collected directly into the blood collection tube containing the anticoagulant solution (Solution B) provided in the kit and that no more than 60-120 µl of blood are collected per 350 µl of anti-coagulant. Blood collection tubes will be capped and inverted several times to mix the blood with Solution B. The blood/Solution B mixture can be stored at room temperature for up to 5 hours before fixing.

The blood will then be fixed using the solution provided in the kit (Solution A). Solution A (fixative) must be kept in an ultracold (–75 to –85 °C) freezer. To prevent Solution A from

excessive warming, the fixation procedure will be performed quickly (less than one minute) and near the ultracold freezer. Immediately prior to fixing, the tube containing the blood sample will be inverted to ensure a homogeneous suspension. Using a pipettor, 180 µl of the blood sample will be transferred to the corresponding labeled 15 ml tube(s) (two replicate samples per rat) containing 2 ml of Solution A. The tube will be recapped securely and vortexed for 3-5 seconds. The tubes will then be immediately transferred to the –75 to –85 °C freezer for storage until analysis by flow cytometry.

On the day of analysis by flow cytometry, the blood samples will be treated with RNase A, labeled with anti-CD71-FITC and anti-CD61-PE, and then stained with the DNA staining solution (propidium iodide). Prior to analysis of samples, the flow cytometer will be set-up and calibrated using the standards (malaria parasite-infected rodent blood stained in parallel with study samples) and protocol supplied in the kit. Anti-CD71-FITC, anti-CD61-PE, and propidium iodide fluorescence signals will be detected in the FL1, FL2, and FL3 channels, respectively. The stop mode will be set so that 20,000 high CD71-expressing cells (immature red blood cells, aka reticulocytes) will be analyzed per sample. The number of CD71-negative cells (mature red blood cells) will also be determined to provide an index of cytotoxicity. Micronucleated reticulocytes will be identified as those that show both CD71 and propidium iodide-associated fluorescence. The data collected from the micronucleus assay will be expressed as the percentage of reticulocytes with micronuclei (%MN-RET) and the percentage of total erythrocytes that were immature (%RET) in each sample.

The test material will be analyzed for purity prior to study initiation. All concentration verification analysis of the dosing solutions and stability analyses will be performed by USACHPPM, Directorate of Laboratory Sciences (DLS), Chromatographic Analysis Division (CAD). Method of analysis will be validated prior to the start of the study.

The study described will be conducted in a manner consistent with the principles of the Good Laboratory Practice (GLP) regulations in the Toxic Substances Control Act (TSCA): 40 CFR (Code of Federal Regulations) 792, plus amendments [10]. The study time frame is expected to be between May and June of 2009.

V.2. <u>Data Analysis</u>: With the exception of clinical chemistry and hematology data, all data will be recorded in the laboratory notebook and/or Labcat. The data from the SSWP will be analyzed according to the methods of Feder et. al.[12, 13] in order to obtain an estimated LD_{50} value, 95% confidence interval, and slope. In accordance with EPA guidelines [18], each dosing group will consist of 10 rats. The data from the 14-day study will be analyzed with a one-way repeated measures ANOVA used to compare the values from the vehicle control group to the values from the treatment groups across observation times. Post hoc comparisons of each treatment group to control animals will be made using Dunnett's test. Daily overt toxicological observations may also be tested using an appropriate categorical test (e.g., Chi square, Fisher's exact) or quantified and evaluated with a one-way ANOVA. Data not normally distributed will be evaluated across treatments with a Kruskal-Wallis One-way ANOVA on ranks (SigmaStat® statistical software,

SPSS Science, Chicago, Ill.). Multiple pairwise comparisons will be completed using the Dunn's Method. For all tests $\alpha = 0.05$ is the level of significance.

Records will be kept in standard USACHPPM laboratory notebooks and/or three ring binders. Daily records will be kept on survival and clinical signs collected on the animals after dosing occurs. Procedures for preparation of any euthanasia solution, drug administration, animal bleeds, observation logs, morbidity/mortality logs, etc... will be stored with the study records. These records will be made available to oversight organizations such as the US EPA, AAALACi, and the IACUC. The protocol, protocol amendments, raw data, statistical analysis, tabular calculations, and graphic analysis of the data will be saved with the study records. Additionally memoranda to the study file, study logs, signature logs, final reports, final report amendments, and test and control articles will be archived at USACHPPM.

V.3. Laboratory Animals Required and Justification:

V.3.1. Non-animal Alternatives Considered: The objective addressed by this study is the adverse health effects of oral exposures of K-26 to the rat. This data will be used to compare the LD_{50} value and the repeated dose LOAEL and NOAEL values for K-26 to that of currently fielded explosives. No non-animal alternative would provide the necessary toxicological information on K-26 to allow for an accurate comparison with previously performed animal testing on other explosives. Therefore, it is necessary to perform these studies in an animal model.

V.3.2. Animal Model and Species Justification: The EPA Health Effects Test Guidelines (OPPTS 870.1100 Acute Oral Toxicity) state that the rat is the preferred species. The Sprague-Dawley rat strain has been historically used for oral toxicity studies in the USACHPPM DTOX and is therefore recommended due to the extensive historical database. Female rats will be used preferentially as they are deemed more sensitive to toxins than males. Different aged animals will be used in the acute and 14 day studies to comply with EPA Guidelines [18]. Older rats (7-12 wks) are used for the acute study because they are fasted overnight before the acute oral dose; fasting causes undue stress to younger rats. Younger rats (4-7 wks) are used for the 14 day study as they will not be fasted, and will require lower doses of K-26 due to their lighter weight.

V.3.3. Laboratory Animals:

V.3.3.1. Genus and Species: *Rattus norvegicus*

V.3.3.2. Strain/Stock: Sprague-Dawley

V.3.3.3. Source/Vendor: Charles River Laboratories (USDA 14-R-0144) or other CHPPM-approved vendor.

V.3.3.4. Age: Acute - 7-12 weeks
14-Day - 4-7 weeks

V.3.3.5. Weight: Various depending on the study. Animals are ordered based on age, not weight range.

V.3.3.6. Sex: Females

V.3.3.7. Special Considerations: None

V.3.4. Number of Animals Required (By Species): 120 rats

V.3.5. Refinement, Reduction, Replacement:

V.3.5.1. Refinement:

No additional refinements will be employed other than the environmental enrichment strategy.

V.3.5.2. Reduction:

1) The SSWP method uses fewer animals than a classic LD_{50} estimation (5 doses, 10 animals per sex per dose) while providing quantitative estimates of median lethality, slope, and confidence intervals.

2) The design of this protocol has expanded the number of experimental groups to be compliant with requirements of the rat micronucleus genotox assay. This eliminates the necessity of running a separate experiment for the sole purpose of conducting a micronucleus assay, dramatically reducing the number of animals consumed.

3) If a dose of 2,000 mg/kg body weight does not cause mortality or morbidity in the first round of acute dosing, then round two of acute dosing will revert to an EPA limit test and thus use fewer animals to estimate an LD_{50}.

4) Only female animals will be used reducing/eliminating consumption of males.

V.3.5.3. Replacement: There is no acceptable methodology available to replace these studies.

V.4. <u>Technical Methods</u>:

V.4.1. Pain/Distress Assessment:

V.4.1.1. APHIS Form 7023 Information

V.4.1.1.1. Number of animals:

V.4.1.1.1.1. Column C: 25

V.4.1.1.1.2. Column D: 35

V.4.1.1.1.3. Column E: 60

V.4.1.2. Pain Relief/Prevention:

V.4.1.2.1. Anesthesia/Analgesia Tranquilization: Anesthesia will be administered prior to cardiac blood collection and euthanasia for the 14-day study. Anesthesia will consist of isoflurane [6]. Isoflurane will be administered with a vaporizer at 3% into a closed box chamber until the rat is unconscious. The rat will then be relocated to a table with a gas nose cone and gas will continue to be administered until the rat is unresponsive to a painful stimulus.

V.4.1.2.2. Pre- and Post-procedural Provisions: Rats will be fasted overnight prior to dosing for the acute portion (Experiment 1) of the study as per EPA Acute Oral Guidelines [2, 18]. The rats will be observed after dosing for acute signs of misadministration, e.g., gasping, respiratory distress, etc., and euthanized when appropriate. The rats will be observed for the 1st 30 minutes after dosing for signs of respiratory distress or immediate toxicity. They will be observed again at the end of the day. The rats will be observed every day of the study, in the morning by the Vet Med Staff and aby the PI or Study Staff at least 4 hr later.

V.4.1.2.3. Paralytics: None

V.4.1.3. Literature Search for Alternatives to Painful or Distressful Procedures:

V.4.1.3.1. Sources Searched:

MEDLINE, TOXLINE, FEDRIP, BIOSIS, EMBASE, CA SEARCH, DTIC, BRD.

V.4.1.3.2. Date of Search: February 13, 2009

V.4.1.3.3. Period of Search: 1950 - 2009

V.4.1.3.4. Key Words of Search: triaminoguanidinium-methyl-5-nitriminotetrazolate, TAG 1-MeATNO2, explosive, toxic, ld50, alternative, welfare, method, model, in vitro, pain, distress, simulate, video, computer, replacement, refinement, reduction

V.4.1.3.5. Results of Search: There were no hits without including the word explosive. This search again returned 20 articles describing *in vivo* toxicity studies on other energetics. No validated *in vitro* tests for acute and 14-day oral toxicity are currently available.

V.4.1.4. Unalleviated Painful/Distressful Procedure Justification:

The nature of the studies precludes the use of totally painless procedures. The toxicity of the compound of interest is unknown and if toxic, the mechanism of toxicity is unknown. An attempt to alleviate pain or distress by the administration of anesthetics, analgesics, or drugs may alter the manifestation of the toxic responses and or interfere with mechanism of toxicity, e.g. non-steroidal anti-inflamatants might mask toxicity of compound caused by induction of systemic inflammation [19]. In addition opioids, such as morphine, have been reported to produce a number of immunomodulatory effects in both laboratory animals and humans. Morphine also

suppresses T- and B-cells, depresses NK activity, and decreases primary antibody responses in mice [20,21]. Animals have been shown to be more susceptible to disease if opioids are used short term or in a single dose [22]. In vitro exposure of murine lymphocytes and macrophages to morphine and its metabolites at a wide range of concentrations resulted in suppressed B cell proliferation, suppression of IL-2, IL-4, and IL-6, and inhibited cytotoxic T lymphocyte induction [23]. Additionally, both fentanyl and buprenorphine (opioids analgesics) have shown a dose dependent attenuation of the serum TNF-α response in mice as a result of exposure to LPS [20]. Previous studies indicate that opioid analgesics, including Butorphanol, an opioid commonly used for pain alleviation in laboratory animals, cause substantial respiratory depression in nonhuman primates [24,25]. Narcotic analgesics can cause histamine release and respiratory depression which could alter the pathogenic and clinical response to infection [26]. Opioids have a significant effect on thermoregulatory control in humans and concentrations of opioids commonly observed in critical care patients significantly inhibit the manifestation of fever [27]. The cyclooxygenase inhibiting NSAIDs have been shown to produce progressive alterations of parameters of the thrombocyte vessel system of hemostasis, decreased ability of thrombocytes to aggregate, and activation of lipid peroxidation processes in rabbits injected with bacterial endotoxin [28]. Steroidal and nonsteroidal anti-inflammatories are contraindicated due to their interference with the inflammatory pathway which is critical in the normal pathogenesis of many physiological processes during the infectious disease process.

a. The observation of the onset, duration and/or reversibility of toxic signs is critical to mechanistic interpretation. "Toxic signs" are defined in TOX SOP 063 [14].

b. Discussions were held with the Attending Veterinarian regarding the painful procedures. The minimal number of animals needed for statistical significance will be used. The final number of rats in each pain category will be reported to the IACUC annually and at the completion of the protocol.

c. To minimize distress, any animal defined as moribund (see V.4.5) will be euthanized with CO_2 in accordance with TOX SOP 066 [3].

V.4.2. Prolonged Restraint: None

V.4.3. Surgery: None

V.4.3.1. Pre-surgical Provisions: Not applicable

V.4.3.2. Procedure: Not applicable

V.4.3.3. Post-surgical Provisions: Not applicable

V.4.3.4. Location: Not applicable

V.4.3.5. Surgeon: Not applicable

V.4.3.6. Multiple Major Survival Operative Procedures:

V.4.3.6.1. Procedures: Not applicable

V.4.3.6.2. Scientific Justification: Not applicable

V.4.4. Animal Manipulations: .

V.4.4.1. Injections: All oral dosing will be administered using an oral gavage needle (16 - 18 ga. x 2 in) using a 1 – 3 ml syringe; maximum volume is not to exceed 10 ml/kg [(2)]. The materials planned for oral administration include the control vehicle, K-26, and ethyl methanesulfonate @ 200 mg/kg (the positive control for the micronucleus assay). If dosing requires administering a volume greater than 10ml/kg, the total dose will be split into 2 smaller volumes that will be given at least 4 hrs apart (4 hrs is the gastric clearance time for the rat).

V.4.4.2. Biosamples: In experiment 2, before isoflurane anesthesia, approximately 100 µl of blood will be collected from the lateral saphenous vein for the micronucleus assay. The hindleg will be extended and immobilized, the skin surface coated with a lubricant (ophthalmic ointment) to aid in visualization of the lateral saphenous vein and to reduce clotting, and the vein punctured with a sterile 16-18 gauge needle.

After isoflurane anesthesia and just prior to euthanasia, a terminal blood draw (approximately 2 - 3 ml) will be taken. The terminal blood sampling will occur after isoflurane gas anesthesia via cardiac puncture using an 18-21 gauge, 1-1.5 inch needle, as outlined in TOX SOP 053 [(14)]. Biosampling will be promptly followed by euthanasia via CO_2.

V.4.4.3. Adjuvants: None

V.4.4.4. Monoclonal Antibody (MAbs) Production: Not applicable

V.4.4.5. Animal Identification: Individual animals will be identified by cage card for the acute study (Experiment 1) and by cage card and subcutaneous transponder for the 14-day study, according to TOX SOP #003 [(15)]. The Principal Investigator and /or study staff will complete the microchipping procedures [(7)].

V.4.4.6. Behavioral Studies: Not applicable.

V.4.4.7. Other Procedures: None.

V.4.4.8. Tissue Sharing: Not applicable.

V.4.5. Study Endpoint: The study endpoint is intervention euthanasia of moribund animals or euthanasia at the end of the two experiments, i.e., 14 days after the studies were initiated. The duration of the observational period for the acute test will not exceed 14 days. Any moribund animal will be euthanized humanely.

One or more of the following clinical signs will be indicative of a moribund animal: impaired ambulation which prevents animals from reaching food or water; excessive weight loss ($\geq$ 20% body weight); lack of physical or mental alertness; prolonged labored breathing; unabated seizure activity; inability to urinate or defecate for greater than 24 hours; or a prolonged inability to remain upright. The Attending Veterinarian may be consulted to evaluate moribund animals and the Attending Veterinarian and PI/SD will determine if euthanasia is indicated for these animals [8].

The time at which signs of toxicity appear, their duration, and the time to death are important, especially if there is a tendency for deaths or morbidity to be delayed. At the end of the observation or dosing period, all surviving animals will be anesthetized for cardiac blood sampling (14-day study), euthanized by CO_2, and necropsied.

V.4.6. Euthanasia: Rats will be euthanized by CO_2 asphyxiation followed by thoracotomy to ensure death in accordance with TOX SOP #066 [3]. Moribund animals will be euthanized with CO_2 in accordance with TOX SOP #066 [3]. The Attending Veterinarian may be consulted if needed to evaluate moribund animals, unless the PI/SD plans to immediately euthanize the animal. The Principal Investigator or Study Staff will perform the euthanasia.

V.5. Veterinary Care:

V.5.1. Husbandry Considerations:

V.5.1.1. Study Room: Studies will be conducted at the USACHPPM Toxicology Directorate facilities, Bldg E-2100 or Bldg E-2101, study room as assigned.

V.5.1.2. Special Husbandry Provisions: Animals will be pair-housed during the acclimatization period for all tests. Animals will be singly housed during study conduct for all tests due to the unknown toxicity of the test substance and food consumption monitoring on the 14-day study. The animals and food containers are weighed and monitored to determine dosing volumes and food consumption. This requires restriction of the food enrichment, i.e., no additional food supplements will be provided other than the food in the food bin.

V.5.1.3. Exceptions: Animals will be singly housed during study conduct for all tests due to the unknown toxicity of the test substance and food consumption monitoring on the 14-day study. No additional food supplements/enrichments will be provided other than the food in the food bin.

V.5.2. Veterinary Medical Care:

V.5.2.1. Routine Veterinary Medical Care: All animals will be observed daily by assigned veterinary medical personnel for husbandry conditions, humane care, and general health [9]. After compound dosing, animals will be observed at least twice daily, in the morning by the veterinary medical personnel and in the afternoon by the Principal Investigator of Study Staff. At least four hours will elapse between observations.

If an animal becomes ill or injured, the observer will report to the Attending Veterinarian and

the PI/SD. The animal will be euthanized by the animal care staff or PI/SD if it becomes critically ill or comatose. Supportive care may be provided by the animal care staff for clinically ill animals if euthanasia is delayed (other than illness associated with the administration of the test compounds).

V.5.2.2. Emergency Veterinary Medical Care: All emergency animal health care will be provided by veterinary medical personnel in consultation with the Principal Investigator whenever possible.

V.5.3. Environmental Enrichment:

V.5.3.1. Enrichment Strategy: All enrichment will be provided in accordance with SOP TOX #122, "Rodent and Rabbit Environmental Enrichment". In addition, the PI/SD, co-investigators, or animal care staff will handle rats several times per week prior to test article exposure to acclimate them to handling prior to study start. Rats may be provided with nylabones in their cage for the duration of all studies. Other enrichment items/activities may be added as approved by the Attending Veterinarian.

V.5.3.2. Enrichment Restriction: Food enrichment will be restricted due to food consumption monitoring. Rodent chow blocks will not be placed on the cage floor for animals during the acclimatization period of the acute study (Experiment 1) due to overnight fasting or during the 14-day study (Experiment 2) due to food consumption monitoring.

VI. STUDY PERSONNEL QUALIFICATIONS AND TRAINING:

Staff Member	Procedure(s)	Training	Years of Experience	Qualifications
Williams, Larry	Handling/Observations Oral Gavage Necropsy Microchipping Bleeding (ic/iv) CO_2 Euthanasia	* Rat handling & injection techniques (CHPPM, 11/03/2008) Necropsy- Rat brain, bone prep, knee joint & sciatic prep (CHPPM, 12/10/2008) TBS TBS TBS	30+ yrs animal research	Ph.D. Anatomy
Adams, Valerie	Handling/Observations Oral Gavage Necropsy Microchipping Bleeding (ic/iv) CO_2 Euthanasia	TBS * Rat handling & injection techniques (CHPPM, 11/03/2008) Necropsy training-recording, weights, & brain removal procedures (CHPPM, 11/05/2008) TBS TBS TBS	7+ yrs animal research	Ph.D. Cell And Structural Biology ALAAS GLP certified

Eck, Bill	Handling/Observations Oral Gavage Necropsy Microchipping Bleeding (ic/iv) CO_2 Euthanasia	Rat handling, gavage training & euthanasia (CHPPM, 7/2007); Rat necropsy training (CHPPM, 12/2007 & 2/2008); AALAS Training online (CHPPM Animal Users' Track) TBS TBS TBS	5+ yrs animal research	PhD Biochemistry
Crouse, Lee	Microchipping Handling/Observations Oral Gavage Bleeding (ic/iv) CO_2 Euthanasia, Necropsy	Rodent Training (WRAIR, 11/1996); Rat handling & gavage training (CHPPM, 7/2007); Humane Care and Use of Lab Animals (5/2000)	15+ yrs animal research.	M.S., Environmental Science
Quinn, Michael	Handling/Observations Oral Gavage CO_2 Euthanasia, Necropsy	Rodent & Small Animal Handling Workshop (MRICD, 6/2005); Rat necropsy training (5/2005, 10/2007)	10+ yrs animal research.	Ph.D., Animal Science
McCain, Wilfred	Handling/Observations Oral Gavage Necropsy	Short course on the care & use of lab animals (5/2000); Animal Care & Use Training (3/1995); Necropsy training (12/2007 & 2/2008)	25+ yrs animal research	Ph.D. Toxicology
LaFiandra, Emily	Bleeding (ic/iv) CO_2 Euthanasia Necropsy	Rodent training (CHPPM, 7/2007); Necropsy training (7/2007 & 10/2007)	10+ yrs animal research	M.S., Wildlife Biology Ph.D., Natural Resources and Environmental Studies
Kilby, Rebecca	Oral Gavage	Rodent & Small Animal Handling Workshop (MRICD, 6/2005, 11/2005); Rat handling & dosing techniques (gavage, injections, euthanasia) (CHPPM 7/2007) Rat handling & injection techniques (CHPPM, 11/03/2008)	10+ yrs animal research	ALAT Certification, MD Licensed Veterinary Technician, AS Veterinary Technology
Hanna, Theresa	Oral Gavage	Rodent & Small Animal Handling Workshop (MRICD, 1998, 2004, 2005); Rat oral gavage training (CHPPM, 10/2004, 3/2008) Rat handling & injection techniques (CHPPM, 11/03/2008)	15+ yrs animal research	ALAT Certification
McFarland, Craig	Handling/Observations Oral Gavage Necropsy	Rodent & Small Animal Handling Workshop (MRICD, 10/2006); Rat handling & gavage training (CHPPM, 7/2007); Rat euthanasia (10/2007)	10+ yrs animal and toxicological investigations	Ph.D., Biology, DVM

TBS = to be scheduled
* - training includes technique of oral gavage

VII. BIOHAZARD/SAFETY: In accordance with CHPPM Regs. 385-1 and 385-5 and TOX SOP 083, standard laboratory protection will be used. Test substances shall be stored in sealed containers when not in use. All manipulations of the test substance, prior to animal treatment, shall be performed in a laboratory (using a fume hood when necessary). Although the precise toxicity of the test substance may not be known, information regarding its chemical family is provided by the sponsor such that a reasonable assessment of its safety can be made [16-18].

VIII. ENCLOSURES:

References (Appendix A).
Support Personnel (Appendix B)

IX. 1 ASSURANCES: As the Principal Investigator on this protocol, I provide the following assurances:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC prior to its implementation.

B. Duplication of Effort: I have made a reasonable, good faith effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

C. Statistical Assurance: I assure that I have consulted with an individual who is qualified to evaluate the statistical design or strategy of this proposal, and that the minimum number of animals determined by EPA guidelines will be used.

D. Biohazard/Safety: I have taken into consideration, and I have made the proper coordinations regarding all applicable rules and regulations regarding radiation protection, biosafety, recombinant issues, and so forth, in the preparation of this protocol.

E. Training: I verify that the personnel performing the animal procedures/manipulations/ observations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures/manipulations.

F. Responsibility: I acknowledge the inherent moral and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to conduct this study in the spirit of the fourth "R", namely "Responsibility," which the DOD has embraced for implementing animal use alternatives where feasible, and conducting humane and lawful research.

G. Scientific Review: This proposed animal use protocol has received appropriate peer scientific review and is consistent with good scientific research practice.

H. Painful Procedures: I am conducting biomedical experiments, which may potentially cause more than momentary or slight pain or distress to animals. This potential pain and/or distress WILL NOT be relieved with the use of anesthetics, analgesics and/or tranquilizers. I have considered alternatives to such procedures; however, I have determined that alternative procedures are not available to accomplish the objectives of this proposed experiment.

Larry R. Williams, Ph.D.

(PRINT) First name, MI, Last name of Study Director/ Principal Investigator

Larry R. Williams 3-26-09

(S.D. Signature) (Date)

IX.2 ASSURANCES: As the Primary Co-Investigator on this protocol, I provide the following assurances:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC prior to its implementation.

B. Duplication of Effort: I have made a reasonable, good faith effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

C. Statistical Assurance: I assure that I have consulted with an individual who is qualified to evaluate the statistical design or strategy of this proposal, and that the minimum number of animals determined by EPA guidelines will be used.

D. Biohazard/Safety: I have taken into consideration, and I have made the proper coordinations regarding all applicable rules and regulations regarding radiation protection, biosafety, recombinant issues, and so forth, in the preparation of this protocol.

E. Training: I verify that the personnel performing the animal procedures/manipulations/ observations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures/manipulations.

F. Responsibility: I acknowledge the inherent moral and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to conduct this study in the spirit of the fourth "R", namely "Responsibility," which the DOD has embraced for implementing animal use alternatives where feasible, and conducting humane and lawful research.

G. Scientific Review: This proposed animal use protocol has received appropriate peer scientific review and is consistent with good scientific research practice.

H. Painful Procedures: I am conducting biomedical experiments, which may potentially cause more than momentary or slight pain or distress to animals. This potential pain and/or distress WILL NOT be relieved with the use of anesthetics, analgesics and/or tranquilizers. I have considered alternatives to such procedures; however, I have determined that alternative procedures are not available to accomplish the objectives of this proposed experiment.

Lee Crouse, M.S.

(PRINT) First name, MI, Last name of Primary Co-Investigator

(S.D. Signature) 3/27/09 (Date)

IX. 3 ASSURANCES: As a Co-Investigator on this protocol, I provide the following assurances:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC prior to its implementation.

B. Duplication of Effort: I have made a reasonable, good faith effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

C. Statistical Assurance: I assure that I have consulted with an individual who is qualified to evaluate the statistical design or strategy of this proposal, and that the minimum number of animals determined by EPA guidelines will be used.

D. Biohazard/Safety: I have taken into consideration, and I have made the proper coordinations regarding all applicable rules and regulations regarding radiation protection, biosafety, recombinant issues, and so forth, in the preparation of this protocol.

E. Training: I verify that the personnel performing the animal procedures/manipulations/ observations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures/manipulations.

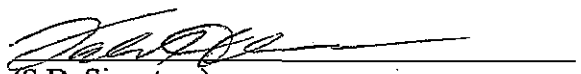
F. Responsibility: I acknowledge the inherent moral and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to conduct this study in the spirit of the fourth "R", namely "Responsibility," which the DOD has embraced for implementing animal use alternatives where feasible, and conducting humane and lawful research.

G. Scientific Review: This proposed animal use protocol has received appropriate peer scientific review and is consistent with good scientific research practice.

H. Painful Procedures: I am conducting biomedical experiments, which may potentially cause more than momentary or slight pain or distress to animals. This potential pain and/or distress WILL NOT be relieved with the use of anesthetics, analgesics and/or tranquilizers. I have considered alternatives to such procedures; however, I have determined that alternative procedures are not available to accomplish the objectives of this proposed experiment.

Valerie Adams, Ph.D..

(PRINT) First name, MI, Last name of Secondary Co-Investigator



(S.D. Signature)

4/20/2009

(Date)

IX.4 ASSURANCES: As a Co-Investigator on this protocol, I provide the following assurances:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC prior to its implementation.

B. Duplication of Effort: I have made a reasonable, good faith effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

C. Statistical Assurance: I assure that I have consulted with an individual who is qualified to evaluate the statistical design or strategy of this proposal, and that the minimum number of animals determined by EPA guidelines will be used.

D. Biohazard/Safety: I have taken into consideration, and I have made the proper coordinations regarding all applicable rules and regulations regarding radiation protection, biosafety, recombinant issues, and so forth, in the preparation of this protocol.

E. Training: I verify that the personnel performing the animal procedures/manipulations/ observations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures/manipulations.

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G. Scientific Review: This proposed animal use protocol has received appropriate peer scientific review and is consistent with good scientific research practice.

H. Painful Procedures: I am conducting biomedical experiments, which may potentially cause more than momentary or slight pain or distress to animals. This potential pain and/or distress WILL NOT be relieved with the use of anesthetics, analgesics and/or tranquilizers. I have considered alternatives to such procedures; however, I have determined that alternative procedures are not available to accomplish the objectives of this proposed experiment.

William Eck, Ph.D.

(PRINT) First name, MI, Last name of Secondary Co-Investigator

William Eck — 3/26/2009

(S.D. Signature) (Date)

IX.5 ASSURANCES: As a Co-Investigator on this protocol, I provide the following assurances:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC prior to its implementation.

B. Duplication of Effort: I have made a reasonable, good faith effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

C. Statistical Assurance: I assure that I have consulted with an individual who is qualified to evaluate the statistical design or strategy of this proposal, and that the minimum number of animals determined by EPA guidelines will be used.

D. Biohazard/Safety: I have taken into consideration, and I have made the proper coordinations regarding all applicable rules and regulations regarding radiation protection, biosafety, recombinant issues, and so forth, in the preparation of this protocol.

E. Training: I verify that the personnel performing the animal procedures/manipulations/ observations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures/manipulations.

F. Responsibility: I acknowledge the inherent moral and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to conduct this study in the spirit of the fourth "R", namely "Responsibility," which the DOD has embraced for implementing animal use alternatives where feasible, and conducting humane and lawful research.

G. Scientific Review: This proposed animal use protocol has received appropriate peer scientific review and is consistent with good scientific research practice.

H. Painful Procedures: I am conducting biomedical experiments, which may potentially cause more than momentary or slight pain or distress to animals. This potential pain and/or distress WILL NOT be relieved with the use of anesthetics, analgesics and/or tranquilizers. I have considered alternatives to such procedures; however, I have determined that alternative procedures are not available to accomplish the objectives of this proposed experiment.

Emily May LaFiandra, Ph.D.

(PRINT) First name, MI, Last name of Secondary Co-Investigator

Emily May LaFiandra — 4/20/2009

(S.D. Signature) (Date)

IX. 6 ASSURANCES: As a Co-Investigator on this protocol, I provide the following assurances:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC prior to its implementation.

B. Duplication of Effort: I have made a reasonable, good faith effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

C. Statistical Assurance: I assure that I have consulted with an individual who is qualified to evaluate the statistical design or strategy of this proposal, and that the minimum number of animals determined by EPA guidelines will be used.

D. Biohazard/Safety: I have taken into consideration, and I have made the proper coordinations regarding all applicable rules and regulations regarding radiation protection, biosafety, recombinant issues, and so forth, in the preparation of this protocol.

E. Training: I verify that the personnel performing the animal procedures/manipulations/ observations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures/manipulations.

F. Responsibility: I acknowledge the inherent moral and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to conduct this study in the spirit of the fourth "R", namely "Responsibility," which the DOD has embraced for implementing animal use alternatives where feasible, and conducting humane and lawful research.

G. Scientific Review: This proposed animal use protocol has received appropriate peer scientific review and is consistent with good scientific research practice.

H. Painful Procedures: I am conducting biomedical experiments, which may potentially cause more than momentary or slight pain or distress to animals. This potential pain and/or distress WILL NOT be relieved with the use of anesthetics, analgesics and/or tranquilizers. I have considered alternatives to such procedures; however, I have determined that alternative procedures are not available to accomplish the objectives of this proposed experiment.

Michael Quinn, Jr., Ph.D.

(PRINT) First name, MI, Last name of Secondary Co-Investigator

(S.D. Signature) 3/26/09 (Date)

APPENDIX A

References

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APPENDIX B

Support Personnel

1. Veterinary Medicine Division:

MAJ Anne MacLarty	Attending Veterinarian
Terry Hanna	Animal Care Technician
Rebecca Kilby	Animal Care Technician
Robert Sunderland	Animal Care Technician

2. Toxicity Evaluation Program:

Dr. Glenn Leach	Biologist
Lee Crouse	Biologist, Co-Investigator
Matthew Bazar	Biologist
Dr. Wilfred McCain	Biologist
Arthur O'Neill	Biologist
Patricia Beall	Biologist
John Houpt	Biologist
Amy Houpt	Biologist
Martha Thompson	Computer Support Specialist

3. Health Effects Research Program:

Dr. Mark Johnson	Toxicologist
Dr. Gunda Reddy	Toxicologist
Dr. Desmond Bannon	Toxicologist
Dr. Craig McFarland	Biologist
Dr. Michael Quinn	Toxicologist, Co-Investigator
Dr. Bill Eck	Biologist, Co-Investigator
Dr. Valerie Adams	Biologist, Co-Investigator
Dr. Emily LaFiandra	Biologist, Co-Investigator
Dr. Larry Williams	Biologist, Study Director

4. Chemistry/Analytical

Curtis Oliver	DLS Chemist
Michael Hable	DLS Chemist

PROTOCOL REVIEW, SUPPORT, APPROVAL SHEET

PROTOCOL NUMBER: 0BME - 30 - 09-03-01 (SUB-JONO / TEST TYPE / IACUC NUMBER)	TITLE: Effects of Oral TAG 1-MeATNO2 (Triaminoguanidinium-1-methyl-5-nitriminotetrazolate - K-26) Exposure to Female Rats (*Rattus norvegicus*)		
1. SCIENTIFIC MERIT (PEER REVIEW)			
1a. Printed Name (First, MI, Last) Desmond Bannon, Ph.D., DABT.	1b. Title Toxicologist, CHPPM-DTOX-HERP	1c. Signature Desmond Bannon	1d. Date (yyyy/mm/dd) 20090224
2. DIRECTOR			
2a. Printed Name (First, MI, Last) LTC Cindy Landgren, Ph.D., DVM.	2b. Title Director, Toxicology	2c. Signature Cindy Landgren	2d. Date (yyyy/mm/dd) 20090227
3. PROGRAM MANAGER			
3a. Printed Name (First, MI, Last) Mark S. Johnson, Ph.D., DABT.	3b. Title Program Manager, Health Effects Research Program	3c. Signature MSJ	3d. Date (yyyy/mm/dd) 20090224
4. ATTENDING VETERINARIAN			
4a. Printed Name (First, MI, Last) MAJ Anne M. MacLarty, DVM, DACLAM	4b. Title Command Animal Programs Manager	4c. Signature MACLARTY.ANNE.MITCHELL.1094566535	4d. Date (yyyy/mm/dd) 20090302
5. ANALYTICAL CHEMISTRY (If Applicable)			
5a. Printed Name (First, MI, Last) for David F Morrow	5b. Title	5c. Signature for David F Morrow	5d. Date (yyyy/mm/dd) 20090223
6. SAFETY MANAGER			
6a. Printed Name (First, MI, Last) Roy Valiant	6b. Title Safety Manager	6c. Signature Roy Valiant	6d. Date (yyyy/mm/dd) 20090223
7. STATISTICIAN (If Applicable)			
7a. Printed Name (First, MI, Last) Robyn Lee	7b. Title Statistician, USACHPPM	7c. Signature Robyn Lee	7d. Date (yyyy/mm/dd) 20090226

PROTOCOL NUMBER: 0BME - 30 - 09-03-01 (SUB-JONO / TEST TYPE / IACUC NUMBER)	TITLE: Effects of Oral TAG 1-MeATNO2 (Triaminoguanidinium-1-methyl-5-nitriminotetrazolate - K-26) Exposure to Female Rats (Rattus norvegicus)		
8. SIO-QAT (GLP COMPLIANCE AND QA SUPPORT)			
8a. Printed Name (First, MI, Last)	8b. Title	8c. Signature	8d. Date (yyyy/mm/dd)
Mike Kefauver	Quality Asssurance Assessor, USACHPPM, Quality Systems Office	KEFAUVER.MICHAEL.P.1229209678	20090224
9. CHAIRMAN, IACUC			
9a. Printed Name (First, MI, Last)	9b. Title	9c. Signature	9d. Date (yyyy/mm/dd)
Jesse Barkley	IACUC Chair	Jesse Barkley	20090323
10. INSTITUTIONAL OFFICIAL			
10a. Printed Name (First, MI, Last)	10b. Title	10c. Signature	10d. Date (yyyy/mm/dd)
Stephen Kistner	Deputy for Technical Services	KISTNER.STEPHEN.L.1228741481	20090330
11. STUDY DIRECTOR/PRINCIPAL INVESTIGATOR			
11a. Printed Name (First, MI, Last)	11b. Title	11c. Signature	11d. Date (yyyy/mm/dd)
Larry R. Williams, Ph.D.	Biologist	WILLIAMS.LAWRENCE.R.1294664404	20090421
12. OTHER ORGANIZATION(S) PROVIDING SUPPORT (AS NEEDED):			
12a. Printed Name (First, MI, Last)	12b. Title	12c. Signature	12d. Date (yyyy/mm/dd)
13. STUDY SPONSOR:			
13a. Printed Name (First, MI, Last)	13b. Title	13c. Signature	13d. Date (yyyy/mm/dd)

USACHPPM PROTOCOL MODIFICATION

For use of this form, see IACUC SOP 1.0

1. DATE: (YYYY/MM/DD) 2009/08/26	2. PROTOCOL NUMBER: 0BME-30-09-03-01	3. MODIFICATION#: 1

4. PROTOCOL TITLE: Effects of Oral TAG 1-MeATNO2 (Triaminoguanidinium-1-methyl-5-nitriminotetrazolate - K-26) Exposure to Female Rats (*Rattus norvegicus*)

5. STUDY DIRECTOR/PRINCIPAL INVESTIGATOR:	6. WORK PHONE:	7. OFFICE SYMBOL:
Larry Williams, Ph.D.	5-7159	MCHB-TS-THE

SECTION I: PREVIOUSLY APPROVED AND CURRENTLY IN USE PROTOCOL MODIFICATIONS

1. MODIFICATION NUMBER	2. SHORT DESCRIPTION OF PRIOR APPROVED MODIFICATION(S)	3. NO. & SPECIES OF ANIMAL REQUESTED	4. APPROVED DATE

SECTION II: CHANGE IN TOTAL # OF ANIMALS USED AND/OR CHANGE IN USDA PAIN CATEGORY

1a. CHANGE: INCREASE TOTAL APPROVED ANIMALS BY: 0 — 1b. N/A ☒

2. ORIGINAL PROTOCOL TOTAL: 120				3. PROTOCOL TOTAL AFTER MODIFICATION: 120			
2a. USDA pain cat:	B: 0	C: 25	D: 35	E. 60	3a. USDA pain cat:	B: 0	C: 25	D: 35	E: 60

4. Yes	No	
☒	☐	Modification requires specific changes or additions to the experimental design of the protocol. (Section V.I. of the template.)
☒	☐	Modification requires changes to the technical methods, i.e., procedures, routes of administration, biosample collection, etc. (Section V.4. of the protocol template.) Indicate training of personnel for new methods, procedures being used.
☐	☒	Modification requires additions or changes in personnel performing procedures. (Section VI of the protocol template.) Include training and qualification information and tasks that each individual will be performing. If changing the Study Director/PI, a signed Assurance Statement needs to be submitted with the modifications.

SECTION III: MODIFICATION/JUSTIFICATION

Explain the modification indicated above in the area below. Indicate any changes in the 3Rs (Refinement, Reduction, Replacement) resulting from changes in number of animals.

PROTOCOL (Page number/para number/line number)	
Section V.1.2.; (page 5, para 1, line 16); Experiment 2	1. MODIFICATION: Replace sentence beginning "Positive control animals ....." with "To produce optimal flow cytometry data, the micronucleus positive control animal group (10 animals) will be orally gavage dosed at three times: on day 1 at time 0, on end of day 1 at time 24 hrs, and on day 2 at time 45 hrs. All positive control animals will be bled, euthanized and necropsied at 48 hours (end of day 2)." This is a change from the original protocol that stated the positive control group would be orally gavaged once per day for the last 2 days of the 14-day study, and then bled, euthanized, and necropsied.
	1a. JUSTIFICATION/REASON: The proposed change in the dose schedule is based on previous experimental data that shows 3 dose time points of the genotoxic agent are required to produce the desired effects, instead of the original 2.

PROTOCOL Page, paragraph, section	Explain the modification indicated above in the area below. Indicate any changes to the 3Rs (Refinement, Reduction, Replacement) resulting from changes (number) of animals used.
Section V.1.2.; (page 5, para 2, line 1); Experiment 2	2. MODIFICATION: Change first sentence to read "On day 14, blood will be collected from the lateral saphenous vein for the micronucleus assay (see below), only from surviving rats in the three highest K-26 dose groups." This is a change from the original protocol that implied all 14-day dosed animals would be anesthetized with isoflurane and bled.
	2a. JUSTIFICATION/REASON: Needed to specify that not all dose groups will be sampled, just animals that survive the 14 days of dosing in the three highest dose groups. All animals from all dose groups will be euthanized with CO2 and undergo necropsy as originally stated in the protocol.
	3. MODIFICATION:
	3a. JUSTIFICATION/REASON:
	4. MODIFICATION:
	4a. JUSTIFICATION/REASON:

Continued on next page YES ☐ NO ☒

SECTION IV. SIGNATURES AND DATES

1. STUDY DIRECTOR: (Printed Name) Larry Williams, Ph.D.	Larry Williams	DATE: (yyyy/mm/dd) 20090826
2. PROGRAM MANAGER:: (Printed Name) Mark S. Johnson		DATE: (yyyy/mm/dd) 2009 0831
3. ATTENDING VETERINARIAN: (Printed Name) Anne M. MacLarty, MAJ, VC	Anne M. MacLarty DVM	DATE: (yyyy/mm/dd) 20090827
4. CHPPM SAFETY OFFICER/OCC HEALTH REP: (IF APPLICABLE) Roy A. Valiant		DATE: (yyyy/mm/dd)
5. CHAIR, IACUC: (Printed Name) APPROVED YES ☑ NO ☐ Jesse J. Barkley	Jesse J Barkley	DATE: (yyyy/mm/dd) 20090831

USACHPPM PROTOCOL MODIFICATION

For use of this form, see DTOX SOP 085

1. DATE: (YYYY/MM/DD) 2009/11/18	2. PROTOCOL NUMBER: 0BME-30-09-03-01	3. MODIFICATION#: 2

4. PROTOCOL TITLE: Effects of Oral TAG 1-MeATNO2 (Triaminoguanidinium-1-methyl-5-nitriminotetrazolate - K-26) Exposure to Female Rats (Rattus norvegicus)

5. STUDY DIRECTOR/PRINCIPAL INVESTIGATOR:	6. WORK PHONE:	7. OFFICE SYMBOL:
Larry Williams, Ph.D.	410-436-7159	MCHB-TS-THE

SECTION I. PREVIOUSLY APPROVED AND CURRENTLY IN USE PROTOCOL MODIFICATIONS

1. MODIFICATION NUMBER	2. SHORT DESCRIPTION OF PRIOR APPROVED MODIFICATION(S)	3. NO. & SPECIES OF ANIMAL REQUESTED	4. APPROVED DATE (XX XXX XXXX
1	Change in dosing schedule for micronucleus positive control group; change in bleed schedule for study animals	N/A	31 Aug 2009

SECTION II. CHANGE IN TOTAL # OF ANIMALS USED AND/OR CHANGE IN USDA PAIN CATEGORY

1a. CHANGE: INCREASE TOTAL APPROVED ANIMALS BY: 0 | 1b. N/A ☒

2. ORIGINAL PROTOCOL TOTAL: 120 | 3. PROTOCOL TOTAL AFTER MODIFICATION: 120

2a. USDA pain cat:	B: 0	C: 25	D: 35	E: 60	3a. USDA pain cat:	B: 0	C: 25	D: 35	E: 60

4. Yes	No	
☒	☐	Modification requires specific changes or additions to the experimental design of the protocol. (Section V.I. of the template.)
☐	☒	Modification requires changes to the technical methods, i.e., procedures, routes of administration, biosample collection, etc. (Section V.4. of the protocol template.) Indicate training of personnel for new methods, procedures being used.
☐	☒	Modification requires additions or changes in personnel performing procedures. (Section VI of the protocol template.) Include training and qualification information and tasks that each individual will be performing. If changing the Study Director/PI, a signed Assurance Statement needs to be submitted with the modifications.

SECTION III. MODIFICATION JUSTIFICATION

Explain the modification indicated above in detail below. Indicate any changes to the 3Rs (Refinement, Reduction, Replacement) as it relates to animal welfare.

PROTOCOL Page, Paragraph, Section	
Page 13, V.5.1.2. Special Husbandry Provisions; V.5.1.3. Exceptions	1. MODIFICATION: Animals will be weighed and implanted with a subcutaneous transponder microchip for identification during their acclimatization period (on Friday 11/20; animals arrived on Wednesday 11/18). This will be an exemption to DTOX SOP 028 (Animal Quality Assurance & Quality Control/Health Monitoring Procedures) allowing the first weight to be taken 2 days after animals arrive and to implant the subcutaneous transponder microchip for identification. Weighing the animals on Friday will be a day -4 weight, not a day -3 weight; this is an exception to the requirement in DTOX SOP 037. The animals will be observed and evaluated via observations and body weights for clinical signs due to distress or health issues (e.g., dehydration, emaciation, respiratory problems, etc.) on Friday prior to implantation. If an animal shows signs of distress or illness, a microchip will not be implanted and will be implanted on Monday or removed from study if still showing signs of distress or illness. Otherwise, the microchip will be implanted per the protocol.
	1a. JUSTIFICATION/REASON: The weighing and microchip implantation procedures need to occur within the 5 day acclimation period that is required by DTOX SOP 028. The 14 day subacute study is scheduled to be conducted over the Thanksgiving holiday and needs to start the week after the animals arrive. Implanting the microchips on Friday will allow the rats to heal over the weekend and recover from the implantation before the daily study dosing begins on Tuesday (11/24).

PROTOCOL Page, Paragraph, Section	Explain the modification indicated above in the area below. Indicate any changes to the 3Rs (Refinement, Reduction, Replacement) resulting from changes in form or # of animals used.
Page 5, V.1.2. Experiment 2: 14-day Repeated Dose Test, paragraph 2; Page 10, V.4.1.2.2. Pre- and Post-Procedural Provisions	2. MODIFICATION: V.1.2.: At the beginning of paragraph 2, add the statement: On day 13 of the study, animals will be fasted overnight, not to exceed 16 hrs, in preparation for the pre-necropsy blood draws scheduled on Day 14. Study staff will be responsible for removing the feed from the animals' cages. 2a. JUSTIFICATION/REASON: Fasted blood samples are required for best results from the clinical analyses (described in protocol).
	3. MODIFICATION: 3a. JUSTIFICATION/REASON:
	4. MODIFICATION: 4a. JUSTIFICATION/REASON:

Continued on next page YES ☐ NO ☒

SECTION IV: SIGNATURES AND DATES

1. STUDY DIRECTOR: (Printed Name) Larry Williams, Ph.D.	Signature Larry T. Williams	DATE: (yyyy/mm/dd) 20091118
2. PROGRAM MANAGER:: (Printed Name) Mark S. Johnson Larry Williams (acting)	Signature Larry T. Williams	DATE: (yyyy/mm/dd) 2009/11/19
3. ATTENDING VETERINARIAN: (Printed Name) Anne M. MacLarty, MAJ, VC	Signature Anne M. MacLarty DVM	DATE: (yyyy/mm/dd) 20091119
4. CHPPM SAFETY OFFICER/OCC HEALTH REP: (IF APPLICABLE) ~~Roy A. Valiant~~ NA	Signature	DATE: (yyyy/mm/dd)
5. CHAIR, IACUC OR QA (If no animal related changes): (Printed Name) Jesse J. Barkley	APPROVED / REVIEWED YES ☒ NO ☐ Signature Jesse W. Barkley	DATE: (yyyy/mm/dd) 20091119

USACHPPM PROTOCOL MODIFICATION

For use of this form, see DTOX SOP 085

1. DATE: (YYYY/MM/DD) 2009/11/20	2. PROTOCOL NUMBER: 0BME-30-09-03-01	3. MODIFICATION#: 3

4. PROTOCOL TITLE: Effects of Oral TAG 1-MeATNO2 (Triaminoguanidinium-1-methyl-5-nitriminotetrazolate - K-26) Exposure to Female Rats (Rattus norvegicus)

5. STUDY DIRECTOR/PRINCIPAL INVESTIGATOR:	6. WORK PHONE:	7. OFFICE SYMBOL:
Larry Williams, Ph.D.	410-436-7159	MCHB-TS-THE

SECTION I. PREVIOUSLY APPROVED AND CURRENTLY IN USE PROTOCOL MODIFICATIONS

1. MODIFICATION NUMBER	2. SHORT DESCRIPTION OF PRIOR APPROVED MODIFICATION(S)	3. NO. & SPECIES OF ANIMAL REQUESTED	4. APPROVED DATE (XX XXX XXXX)
1	Change in dosing schedule for micronucleus assay positive control group; change in bleed schedule for study animals	N/A	31 Aug 2009
2	Weigh and microchip during acclimatization period exception to SOP 028; day -4 weight exception to SOP 037; overnight fast before bleed and euthanasia	N/A	19 Nov 2009

SECTION II. CHANGE IN TOTAL # OF ANIMALS USED AND/OR CHANGE IN USDA PAIN CATEGORY

1a. CHANGE: INCREASE TOTAL APPROVED ANIMALS BY: 0 — 1b. N/A ☒

2. ORIGINAL PROTOCOL TOTAL: 120					3. PROTOCOL TOTAL AFTER MODIFICATION: 120				
2a. USDA pain cat:	B: 0	C: 25	D: 35	E: 60	3a. USDA pain cat:	B: 0	C: 25	D: 35	E: 60

4. Yes	No	
☐	☒	Modification requires specific changes or additions to the experimental design of the protocol. (Section V.I. of the template.)
☒	☐	Modification requires changes to the technical methods, i.e., procedures, routes of administration, biosample collection, etc. (Section V.4. of the protocol template.) Indicate training of personnel for new methods, procedures being used.
☐	☒	Modification requires additions or changes in personnel performing procedures. (Section VI of the protocol template.) Include training and qualification information and tasks that each individual will be performing. If changing the Study Director/PI, a signed Assurance Statement needs to be submitted with the modifications.

SECTION III. MODIFICATION JUSTIFICATION

Explain the modification indicated above in the area below. Indicate any changes to the 3Rs (Refinement, Reduction, Replacement) resulting from changes in number of animals.

PROTOCOL Page, Paragraph, Section	
p. 10, Section V.4.1.2.2.	1. MODIFICATION: Delete the last two sentences beginning with: "They will be observed again..." and replace with "They will be observed again approximately 4 hours or later following dosing with the possible exception of non-duty days (weekends / holidays) during the 14-day sub-acute study based on a weight-of-evidence approach and in consultation with the AV (See Section V.5.2.1.)."
	1a. JUSTIFICATION/REASON: Animals in the acute study showed no signs of toxicity at the limit dose of 2000 mg/kg. Doses in the sub-acute study will not be above 2000 mg/kg. If observations during the beginning of the sub-acute study and the 30-minute post-dose observations on the day of consultation indicate a general lack of toxicity and other adverse clinical signs, and there are no distressed animals likely to require some form of intervention in less than a 24-hour period, the 4-hour post-dose observations on non-duty days can be justified as not critical for animal welfare given the frequency of duty day observations. The exception is also scientifically justified since it will not affect the endpoints of this particular study.

PROTOCOL Page, paragraph & Section	Explain the modification indicated above in the area below. Indicate any changes to the 3R's (Refinement, Reduction, Replacement) resulting from changes in number of animals used.
p. 13, Section V.5.2.I., line 42	2. MODIFICATION: Delete the second and third sentences beginning with: "After compound dosing..." and replace with the following: "Animals will be observed at least twice daily on duty days and at least once daily on non-duty days by assigned Veterinary Medicine personnel and/or study staff. Observations by study staff will be taken approximately 4 hours or later following dosing. However, a weight-of-evidence approach in consultation with the AV will be used during the 14-day sub-acute study to determine the continued necessity of 4-hour post-dose observations on non-duty days. The weight-of (continued on attachment)
	2a. JUSTIFICATION/REASON: Animals in the acute study showed no signs of toxicity at the limit dose of 2000 mg/kg. Doses in the sub-acute study will not be above 2000 mg/kg. If observations during the beginning of the sub-acute study and the 30-minute post-dose observations on the day of consultation indicate a general lack of toxicity and other adverse clinical signs, and there are no distressed animals likely to require some form of intervention in less than a 24-hour period, the 4-hour post-dose observations on non-duty days can be justified as not critical for animal welfare given the frequency of duty day observations. The exception is also scientifically justified since it will not affect the endpoints of this particular study.
	3. MODIFICATION: **This observation schedule based on weight-of-evidence is unique and specific to this protocol.
	3a. JUSTIFICATION/REASON:
	4. MODIFICATION:
	4a. JUSTIFICATION/REASON:

Continued on next page YES ☐ NO ☒

SECTION IV - SIGNATURES AND DATES

1. STUDY DIRECTOR: (Printed Name) Larry Williams, Ph.D.	Signature Larry A. Williams	DATE: (yyyy/mm/dd) 20091120
2. PROGRAM MANAGER:: (Printed Name) Mark S. Johnson	Signature Desmond Banks (for M.S.)	DATE: (yyyy/mm/dd)
3. ATTENDING VETERINARIAN: (Printed Name) Anne M. MacLarty, MAJ, VC	Signature Anne M. MacLarty, DVM	DATE: (yyyy/mm/dd) 20091123
4. CHPPM SAFETY OFFICER/OCC HEALTH REP: (IF APPLICABLE) Roy A. Valiant	Signature	DATE: (yyyy/mm/dd)
5. CHAIR, IACUC OR QA (If no animal related changes): (Printed Name) Jesse J. Barkley / Robyn Lee Alternate	APPROVED REVIEWED YES ☒ NO ☐ Signature	DATE: (yyyy/mm/dd) 2009/11/24

Modification Item 2, continued from CHPPM Form 28-R-E:

p.13, Section V.5.2.1.

...-evidence will consist of results from the acute study, observations from at least the first 4-5 days of the sub-acute study, and the 30-minute post-dose observations on the day of consultation. If the evidence suggests a general lack of toxicity and other adverse clinical signs, and there are no distressed animals likely to require some form of intervention in less than a 24-hour period, the 4-hour post-dose non-duty day observations will be considered optional. It is recognized that the potential for cumulative effects exists and such observations may be necessary toward the end of the sub-acute study. Observations by study personnel will be documented in the study records. It will be noted in the room log book when the animals have been observed and an entry will be made for animals showing potential signs of toxicity or distress."

USACHPPM PROTOCOL MODIFICATION

For use of this form, see DTOX SOP 085

1. DATE: (YYYY/MM/DD) 2009/11/17	2. PROTOCOL NUMBER: 0BME-30-09-03-01	3. MODIFICATION#: GLP-1

4. PROTOCOL TITLE: Effects of Oral TAG 1-MeATNO2 (Triaminoguanidinium-1-methyl-5-nitriminotetrazolate - K-26) Exposure to Female Rats (Rattus norvegicus)

5. STUDY DIRECTOR/PRINCIPAL INVESTIGATOR:	6. WORK PHONE:	7. OFFICE SYMBOL:
Larry Williams, Ph.D.	5-7159	MCHB-TS-THE

SECTION I: PREVIOUSLY APPROVED AND CURRENTLY IN USE PROTOCOL MODIFICATIONS

1. MODIFICATION NUMBER	2. SHORT DESCRIPTION OF PRIOR APPROVED MODIFICATION(S)	3. NO. & SPECIES OF ANIMAL REQUESTED	4. APPROVED DATE (XX XXX XXXX

SECTION II: CHANGE IN TOTAL # OF ANIMALS USED AND/OR CHANGE IN USDA PAIN CATEGORY

1a. CHANGE: INCREASE TOTAL APPROVED ANIMALS BY: 0					1b. N/A ☒				
2. ORIGINAL PROTOCOL TOTAL: 120					3. PROTOCOL TOTAL AFTER MODIFICATION: 120				
2a. USDA pain cat:	B: 0	C: 25	D: 35	E: 60	3a. USDA pain cat:	B: 0	C: 25	D: 35	E: 60

4. Yes	No	
☒	☐	Modification requires specific changes or additions to the experimental design of the protocol. (Section V.I. of the template.)
☐	☒	Modification requires changes to the technical methods, i.e., procedures, routes of administration, biosample collection, etc. (Section V.4. of the protocol template.) Indicate training of personnel for new methods, procedures being used.
☐	☒	Modification requires additions or changes in personnel performing procedures. (Section VI of the protocol template.) Include training and qualification information and tasks that each individual will be performing. If changing the Study Director/PI, a signed Assurance Statement needs to be submitted with the modifications.

PROTOCOL Page, paragraph, section	SECTION III: MODIFICATION JUSTIFICATION *Explain the modification indicated above in the checkbox. Indicate any changes to the 3Rs (Refinement, Reduction, Replacement resulting from changes in number of animals).*
page 5,, Section V.1.2, Experiment 2, Paragraph 2	1. MODIFICATION: Change "The following tissues will be harvested and weighed: brain, heart, kidneys, adrenals, lungs, liver, spleen, and thymus." to Read "The following tissues will be harvested and weighed: brain, heart, kidneys, adrenals, lungs, liver, spleen, ovaries, uterus, and thymus."
	1a. JUSTIFICATION/REASON: The rats in the study are female and I ommitted the inclusion of sex organs in the original protocol. We need to collect these tissues and weigh them so the ovaries and uterus need to be added to the GLP portocol.

PROTOCOL Page, paragraph, section	Explain the modification indicated above in the area below. Indicate any changes to the 3Rs (Refinement, Reduction, Replacement) resulting from changes in number of animals used.
	2. MODIFICATION:
	2a. JUSTIFICATION/REASON:
	3. MODIFICATION:
	3a. JUSTIFICATION/REASON:
	4. MODIFICATION:
	4a. JUSTIFICATION/REASON:

Continued on next page YES ☐ NO ☒

SECTION IV. SIGNATURES AND DATES

1. STUDY DIRECTOR: (Printed Name) Dr. Lawrence R. Williams	Signature	DATE: (yyyy/mm/dd) 20091118
2. PROGRAM MANAGER:: (Printed Name) N/A	Signature	DATE: (yyyy/mm/dd)
3. ATTENDING VETERINARIAN: (Printed Name) N/A	Signature	DATE: (yyyy/mm/dd)
4. CHPPM SAFETY OFFICER/OCC HEALTH REP: (IF APPLICABLE) N/A	Signature	DATE: (yyyy/mm/dd)
5. CHAIR, IACUC OR QA (If no animal related changes) (Printed Name) Michael P. Kefauver, Quality Assurance, Quality Systems Office	APPROVED / REVIEWED YES ☒ NO ☐ Signature	DATE: (yyyy/mm/dd) 20091118

USACHPPM PROTOCOL MODIFICATION

For use of this form, see DTOX SOP 085

1. DATE: (YYYY/MM/DD) 2010/01/12	2. PROTOCOL NUMBER: 0BME-30-09-03-01	3. MODIFICATION#: GLP-2

4. PROTOCOL TITLE: Effects of Oral TAG I-MeATN02 (Triaminoguanidinium-l-methyl-5-nitriminotetrazolate - K-26) Exposure to Female Rats (Rattus norvegicus)

5. STUDY DIRECTOR/PRINCIPAL INVESTIGATOR:	6. WORK PHONE:	7. OFFICE SYMBOL:
Larry Williams, Ph.D	5-7159	MCHB-TS-THE

SECTION I. PREVIOUSLY APPROVED AND CURRENTLY IN USE PROTOCOL MODIFICATIONS

1. MODIFICATION NUMBER	2. SHORT DESCRIPTION OF PRIOR APPROVED MODIFICATION(S)	3. NO. & SPECIES OF ANIMAL REQUESTED	4. APPROVED DATE (XX XXX XXXX
GLP-1	Change "The following tissues will be harvested and weighed: brain, heart, kidneys, adrenals, lungs, liver, spleen, and thymus"	0	17 Nov 2009

SECTION II. CHANGE IN TOTAL # OF ANIMALS USED AND/OR CHANGE IN USDA PAIN CATEGORY

1a. CHANGE: INCREASE TOTAL APPROVED ANIMALS BY: 0 — 1b. N/A ☒

2. ORIGINAL PROTOCOL TOTAL: 120 — 3. PROTOCOL TOTAL AFTER MODIFICATION: 120

2a. USDA pain cat:	B: 0	C: 25	D: 35	E. 60	3a. USDA pain cat:	B: 0	C: 25	D: 35	E: 60

4. Yes	No	
☒	☐	Modification requires specific changes or additions to the experimental design of the protocol. (Section V.I. of the template.)
☐	☒	Modification requires changes to the technical methods, i.e., procedures, routes of administration, biosample collection, etc. (Section V.4. of the protocol template.) Indicate training of personnel for new methods, procedures being used.
☐	☒	Modification requires additions or changes in personnel performing procedures. (Section VI of the protocol template.) Include training and qualification information and tasks that each individual will be performing. If changing the Study Director/PI, a signed Assurance Statement needs to be submitted with the modifications.

SECTION III. MODIFICATION JUSTIFICATION

Explain the modification indicated above (in the area below). Indicate any changes to the 3R's (Refinement, Reduction, Replacement) resulting from changes in number of animals.

PROTOCOL Page, paragraph, section	
Page 5, Section V.1.2 (Experiment 2: 14-Day Repeated Dose Test), paragraph 2, at the end of the paragraph	1. MODIFICATION: To Page 5, Section V.1.2 (Experiment 2: 14-Day Repeated Dose Test), paragraph 2, at the end of the paragraph, add the statement "If the results of the gross necropsy are suggestive of adverse effects, a gross section/sections of the effected tissues will be collected, weighed, preserved in 10% formalin, and sent for histopathology:" For this study, the following cross section/sections were collected and will be sent for histopathology examination: 2 sections of liver and the left kidney for 5 - controls; 5 from 500 mg/kg; 5 from 1000 mg/kg and 5 from 2000 mg/kg.
	1a. JUSTIFICATION/REASON: During the gross necropsy some adverse effects were observed in certain tissues and a cross section/section was collected, weighed, preserved in 10 % formalin. Furthermore, the tissues listed (section III.1 above) will be sent for histopathology examination. This is an addition to the original protocol.

PROTOCOL Page, para., sph section	*Explain the modification indicated above in the area below. Indicate any changes to the 4R's (Refinement, Reduction, Replacement) resulting from changes in number of animals used.*
	2. MODIFICATION:
	2a. JUSTIFICATION/REASON:
	3. MODIFICATION:
	3a. JUSTIFICATION/REASON:
	4. MODIFICATION:
	4a. JUSTIFICATION/REASON:

Continued on next page YES ☐ NO ☒

SECTION IV. SIGNATURES AND DATES

1. STUDY DIRECTOR: (Printed Name) Larry Williams, Ph.D	Signature Larry Williams	DATE: (yyyy/mm/dd) 1-13-10
2. PROGRAM MANAGER:: (Printed Name) N/A	Signature	DATE: (yyyy/mm/dd)
3. ATTENDING VETERINARIAN: (Printed Name) N/A	Signature MPK 1/13/10 Recording ERROR	DATE: (yyyy/mm/dd)
4. CHPPM SAFETY OFFICER/OCC HEALTH REP: (IF APPLICABLE) N/A	Signature	DATE: (yyyy/mm/dd)
5. CHAIR, IACUC OR QA (If no animal related changes): (Printed Name) Michael P. Kefauver, Quality Assurance, Quality Systems Office	APPROVED (REVIEWED) YES ☑ NO ☐ Signature Michael P. Kefauver	DATE: (yyyy/mm/dd) 2010/01/13

LABORATORY ANIMAL ISSUE/TURN-IN FORM

For use of this form, see DTOX SOP 004

DATE: *(yyyy/mm/dd)* 2009/09/30 ☒ FACILITY RECEIPT AND ISSUE TO PROTOCOL and/or ☒ TURN-IN/TRANSFER

SECTION I - IDENTIFICATION INFORMATION

1. SOURCE/VENDOR: Charles River Laboratories

2. SHIPMENT/LOT # 30977548 / 171

3. SHIPMENT RECEIVE DATE: *(yyyy/mm/dd)* 2009/09/30

4. QUARANTINE DATES: 30 Sept 2009 - 4 Oct 2009

5. MICROCHIP/ID #(s) IF APPLICABLE):

6. SPECIES:	7. SEX:	8. TOTAL:
Rats- Sprague-Dawley	0 MALE 30 FEMALE	30

SECTION II

1. FOR FACILITY RECEIPT/ISSUE:

a. ISSUE TO PROJECT & PROTOCOL # 0BME-30-09-03-01 b. PROTOCOL APPROVAL DATE: 23 March 2009

c. STUDY DIRECTOR (SD)/PRINCIPAL INVESTIGATOR (PI): Larry Williams

d. PROTOCOL TITLE:

Effects of Oral TAG 1-MeATNO2 (Triaminoguanidinium-1-methyl-5-nitriminotetrazolate- K-26) Exposure to Female Rats (Rattus norvegicus)

e. ANIMALS MAY BE PLACED ON STUDY: 2009 Oct 5 (DATE: yyyy/mm/dd)

2. FOR TRANSFER/TURN-IN:

a. FROM PROTOCOL # 0BME-30-09-03-01 b. SD: Larry Williams c. QUANTITY: 5

d. TO PROTOCOL # 09-03-02 e. SD; MAJ Anne MacLarty

f. ANIMAL ID NUMBER(s):

R09-0850, R09-0851, R09-0852, R09-0853, R09-0854

3. OTHER ACTION:

PART A - FROM HERE DOWN WILL BE ENTERED BY HAND

4. HEALTH STATUS AT TIME OF RECEIPT (ISSUE) OR TRANSFER (VETERINARIAN):		5. SIGNATURE OF ATTENDING VETERINARIAN:	6. DATE:
Healthy		Anne M MacLarty DVM	30 Sep 09
7. SIGNATURE OF ISSUER: (VETERINARIAN OR ANIMAL CARE STAFF)	8. DATE:	9. SIGNATURE OF RECEIVER:	10. DATE:
Rebecca Ki	30 Sep 09		30 Sept 09
11. TURN-IN/TRANSFER: (Signature of person turning in)	12. DATE:	13. TURN-IN/TRANSFER:	14. DATE:
	10-29-09	Anne M MacLarty DVM	29 Oct 09

NOTES: Animal ID#'s R09-0825 thru R09-0854

NOTE: IF ANIMALS ARE BEING TRANSFERRED FROM ONE PROTOCOL TO ANOTHER, THE ANIMALS WILL NOT BE USED BY THE NEW SD UNTIL THE INITIA SD HAS APPROVED THE TRANSFER.